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[illegible]OST ON WHEAT AT PROGRESSIVE STAGES OF MATURITY

[illegible]TION AND BIOCHEMICAL PROPERTIES OF GRAIN AND FLOUR[1]

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Abstract

The increase in dry weight of wheat kernels after flowering accelerated slightly for 14 days, was steady for 10 days, then fell off gradually to zero in about 14 days more, when the kernels weighed 32 gm. per 1,000. At this stage they contained 58 to 60% dry matter, a critical point marking the practically complete cessation of chemical changes.

The weight of nitrogen increased steadily to about 1 gm. per 1,000 kernels at the critical stage. Respiration losses of carbon, before and after harvest, appear to account largely for the changes in the percentage nitrogen in the kernels. The ratio of nitrogenous to non-nitrogenous material moved into the endosperm seems to have been nearly constant throughout the main developmental period.

Ammonia nitrogen first increased to a maximum of 4.8 mg. per 1,000 kernels, then decreased to negligible proportions at the critical stage. Salt-soluble nitrogen in fresh kernels decreased from an initial value of 75% of the total nitrogen to 22% at the critical stage, when a little more than one-third of it was non-protein. Drying the kernels before analysis changed the percentage composition, owing to respiration and synthesis, by an amount varying with rate and conditions of drying.

Frost had no effect on the ash content of the kernels. Four degrees of frost (28° F.) had no effect on the per cent total, salt-soluble or non-protein nitrogen, but 8, 10 and 14 degrees, in cuttings before the critical stage, in both the grain and the flour milled from it reduced the per cent total nitrogen, an effect ascribed to slowing up of respiration, and increased the per cent of the fractions, ascribed to checking of synthesis.

Yields of washed gluten from the control samples were about the same at all stages, but physical properties did not attain maximum quality before the critical stage. Four degrees of frost did not affect yield, but reduced quality in cuttings before the critical stage. More severe frost reduced both yield and quality in immature samples, the effect of the heaviest frost on quality persisting to full maturity.

Both reducing and invert sugar percentages declined in early stages of development. Frost did not affect invert sugar content, but 8, 10 and 14 degrees increased reducing sugars in flours from grain cut before the critical stage. This is ascribed partly to increased enzyme activity, as indicated by greater maltose production, and partly to slowing of respiration by frost injury. Gain in kernel weight by translocation after cutting took place in immature check samples, but not in heavily frozen samples. Respiration losses in the stook were calculated to be about twice as great from the checks as from the heavily frozen samples.

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Introduction

The first paper of this series (18) dealt with the physica wheat kernels from plants which had been subjected to grade at progressive stages of maturity in the seasons of 1929, 193 the present paper, the composition and certain biochemical grain and flour from the 1930 samples are described.

Previous Work

The study of the chemical development of wheat and the effe[illegible]ost on this development has received most attention during the last twe[illegible] years or so, but there are publications on the subject dating back at least to 1866. The earliest papers to which reference has been found were published by French workers at about this time. Most of the earlier literature dealing with frozen wheat and appearing from 1885 to 1900 is concerned with the feeding value of the frozen grain, but the analyses are of interest. Only a selected group of papers bearing directly on the present investigations will be reviewed.

Fresh and dry weight. The percentage moisture content of wheat kernels has been found to decrease more or less regularly from the time of flowering until maturity, but the actual weight of water has been found to increase at first, soon reaching a roughly constant level, and then to drop rapidly (5, 24, 34). Brenchley and Hall (5) found that the constant-weight period was about 18 days long, while Woodman and Engledow (34) reported it as 10 days. The latter workers further determined that the fresh weight of the kernels increased until rapid desiccation set in, when a distinct change in kernel color from green to yellowish-brown could be noted. The weight of dry matter increased until the same time, but from then on changed little until the final week, during which period a measurable decrease was recorded. The fact that the changes in weight of dry matter are slight after the dry matter reaches 56–60% of the kernel weight has been substantiated by the work of Arny and Sun (1), Olson (20), Saunders (22, 23) and Sharp (24). Arny and Sun, however, report that although changes are slight after this stage has been reached, there is a gradual rise until maturity, but that translocation of materials from the vegetative parts to the kernel ceases as soon as the grain is cut. The latter observation is supported by the work of Wilson and Raleigh (33). Shutt (27) and Saunders (23), in a joint experiment, found that the weight per 1,000 kernels of wheat dried in the stook was higher than that of wheat removed from the heads before drying, in cuttings up to the "firm dough" stage, indicating that there must have been translocation of food materials after cutting. At later stages the relation was slightly reversed.

Total nitrogen. Early workers reported that the percentage total nitrogen in wheat kernels decreased as the grain developed, explaining this by the theory that a larger proportion of the nitrogenous materials is deposited in early stages, the later filling consisting mainly of starch. Dehérain and Dupont (8) stated that the nitrogenous matter is almost completely elaborated by the time maturation commences, while the starch accumulates in the grain during the last weeks. To explain how starch accumulation could be possible when the only part of the plant remaining green was the upper part of the stem, they demonstrated by laboratory experiments that this part was still capable of actively assimilating carbon from the atmosphere, while by cutting the heads from certain plants at this stage in the field and analyzing the top 15 cm. of the stems 24 hr. later, in comparison with stems of plants left intact, they demonstrated a large accumulation of starch in the stems without heads, with no change in the intact stems. In neither kind of stem was there any change in sugar or nitrogen content. They concluded, therefore, that nitrogen is not being elaborated or translocated at that stage but that the deposition of starch in the kernels is proceeding rapidly. The experimental results obtained by Kedzie (13), and more recently by Arny and Sun (1) and Olson (20) also indicate that there is a fall in percentage total nitrogen during the developmental period.

Most of the modern work is in disagreement with these results, revealing instead that although there is an initial decrease in percentage total nitrogen, this decrease does not long persist, and that a distinct rise occurs toward the end of the developmental period. The amounts of the early decrease and later increase have been found to vary considerably in separate experiments, but the fairly consistent reports of this relation seem to justify the belief in its existence.

Brenchley and Hall (5) described such a course of events, explaining that the pericarp tissues, which are formed first, are rich in nitrogen and that the percentage of nitrogen in the kernels consequently decreases as the less nitrogeneous endosperm is moved in, though the composition of the latter appeared constant during the main filling period. They ascribed the final rise in percentage nitrogen to losses of carbon by respiration. Thatcher (29, 30) accepted this general view, but doubted that respiration could account entirely for the rise in the percentage nitrogen he found in the last stages of kernel development. Woodman and Engledow (34) found the same sequence with kernels analyzed in the fresh condition, as did Saunders (22, 23) with dried material, though in one of his series the ripe sample did not attain the value of the original.

Beginning with Brenchley and Hall (5), various workers have reported their results not only on a percentage basis, but also on the basis of the actual weight of nitrogen and protein in the developing kernels. Saunders (22) found that the protein content per 1,000 kernels rose from 0.884 to 5.104 gm. during development, the rise being most rapid during the early stages, and that the rate of deposition of other materials followed much the same course. Thatcher (29, 30) found similar changes in protein, but a decreasing ratio of carbohydrates to protein after the milk stage.

The effect of respiration on the total nitrogen content of wheat has been studied by McGinnis and Taylor (17) and Tottingham (31). The former workers measured the evolution of carbon dioxide from wheat kernels collected daily, and calculated that about 10 gm. of carbohydrate material per 100 gm. dry matter was used up by respiration during a 16-day ripening period, or sufficient to cause an increase of 1.46% in the protein content of the wheat, unless compensated by fresh carbohydrate material moved into the kernels. While the respiration of threshed kernels may not reflect accurately what occurs in the field, such results do indicate that the loss of carbohydrates is relatively great, perhaps great enough to explain the actual rise found in protein content of wheat under normal conditions. Tottingham found that carbohydrate losses were largely influenced by temperature. Since the ratio of respiration to synthesis was greater at higher temperatures than at lower, the grain grown at the higher temperatures contained a higher percentage protein than that grown under cooler conditions, notwithstanding that the actual amount of protein synthesized was less at the higher temperatures.

Shutt (26) and Harcourt (11) reported that frozen wheat was higher in total nitrogen than non-frozen and, in the belief that proteins are deposited in the kernels earlier than starch, attributed this to the immaturity of the wheat when frozen. The data given in their reports, however, do not appear adequate to support the conclusion that frozen wheat is actually higher in nitrogen. Sharp (25) found the reverse situation during the early stages of kernel development, but after the kernels reached a dry matter content of about 54% there were no differences. Johnson and Whitcomb (12) conclude from their own work and from a survey of the literature that differences in percentage nitrogen content of frozen and non-frozen wheat are due to factors other than frost. They also found that the total nitrogen content of flour milled from frozen wheat bore a normal relation to that of the wheat. Sharp (25) on the other hand, found some of the flours from his frozen samples abnormally high in total nitrogen. This he ascribed in part to milling difficulties with immature wheat, but not entirely, since some flours were higher in nitrogen than the wheat from which they were milled. When the kernels contained 54% or more dry matter at the time of freezing, however, the relation between the nitrogen content of wheat and flour was normal.

Salt-soluble nitrogen. Woodman and Engledow (34) found the percentage of nitrogen soluble in 1% sodium chloride decreased in the developing kernels until these had reached the stage at which they contained about 55% dry matter, and from this point on remained fairly constant. Sharp (24) found that frozen wheat, especially those samples which were immature when frozen, contained higher percentages of nitrogen soluble in 5% potassium sulphate than did the unfrozen check samples.

Non-protein nitrogen. According to Woodman and Engledow (34) the non-protein nitrogen in wheat follows a very similar course to that of the salt-soluble nitrogen except that the amounts are smaller. There was very little change during the last few days, and the fact that any of these lower compounds remained was ascribed to trapping of them in the rapidly developing tissues where they had no chance of taking part in synthetic processes. These lower nitrogen compounds are reported by Shutt (26), Blish (3), and Johnson and Whitcomb (12) to be present in larger amounts in frozen than in non-frozen wheat, the largest differences being found in the most immature grain. Johnson and Whitcomb suggest that when synthesis has been stopped by frost the non-utilized compounds persist in the dried kernels, while in non-frozen material at a similar stage of development synthesis proceeds to a much greater extent during the drying process. Shutt (26) reported that when frozen and non-frozen wheat was milled, these differences in non-protein nitrogen were not observed in the flour, but Blish (3) and Sharp (24) found the differences still persisted, especially in the more immature samples.

Gluten. Woodman and Engledow (34) report that the most immature fresh kernels showing distinct gluten formation contained 55% dry matter at the time of cutting. The amount of gluten increased as the grain became riper. Crude gluten determinations have been made on frozen and check samples by Whitcomb and Sharp (32) and Johnson and Whitcomb (12). In immature samples they found the dry gluten percentage higher in the checks than in the frozen material, but in all wheat which had reached a dry matter content of 54–56% before it was frozen the value was unchanged by the frost. Whitcomb and Sharp found that in every sample analyzed the percentage dry gluten was less than the percentage crude protein calculated as total nitrogen × 5.7. Thatcher (28) and Olson (19) had early shown that the relation varied with the strength of the flour, weak glutens losing more by dispersion in washing, while Dill and Alsberg (9) more recently showed the importance of the electrolyte content of the wash water. The ratio of gluten to crude protein has no absolute basis or meaning, as neither substance is pure protein. Gluten varies in composition with the method of washing, while crude protein not only varies in composition with maturity and other factors in the wheat, but its quantity is calculated from the nitrogen content by an arbitrary factor upon the size of which not all chemists agree. The ratio is nevertheless of some industrial interest, both factors being widely used in evaluating flour strength.

Glutenin and gliadin. Recent work in this laboratory (6) and elsewhere (4) has cast doubt on the chemical entity of glutenin and gliadin as separated from gluten by any of the methods so far proposed. A series of determinations of glutenin in the course of the investigations to be reported in this paper showed such unsatisfactory agreement among the three published methods tried, that the results were discarded and the determination discontinued. Though several of the papers already cited in this review give results for glutenin and gliadin, discussion of them does not seem to be justified at present, because of the doubt as to the validity of the methods employed.

Sugars. Brenchley and Hall (5) report the "sugar (dextrose)" content of their earliest samples of wheat kernels, taken about 10 days after flowering, as about 15% of the dry matter, falling rapidly during the course of three weeks to about 2%, at which figure it remained fairly constant for the last fortnight before cutting. By "dextrose" the authors may mean total sugar calculated as dextrose, since when reducing sugar has been reported separately by various authors the quantity has usually been a relatively small fraction of the total sugar, the latter being more of the order of the figures given above. The actual weight of sugar per 1,000 kernels, calculated by Brenchley and Hall, increased during the first few samplings, before dropping to an approximately constant level. These trends agree with those found later by Thatcher (30) who, however, did not begin sampling early enough to note the initial rise in weight.

Blish (3) states that the percentage of reducing sugars is greater in frozen than in sound wheat, and that it increases with the severity of freezing. The data he presents do not, however, separate the effects of immaturity and frost, but rather let it appear that the former was the major influence. The percentage of sucrose was relatively unaffected by either factor. Johnson and Whitcomb (12) also report the combined effect of immaturity and frost on the content of reducing sugar, which varied from 0.09% in flour from normal wheat to 15.04% in flour from the most immature, frosted wheat.

Diastatic activity. During the first fortnight of their sampling period, which began about 10 days after flowering, Brenchley and Hall (5) found the diastatic power of the fresh grain per 100 gm. dry matter, increased, after which it fell steadily. Recalculating their results to show diastatic power per 1,000 grains, they found it remained about constant after the initial rise. Mangels and Stoa (15) and Malloch (14) found a tendency for the diastatic activity of flour to decrease with advancing maturity of the grain from which it was milled. Their series, however, covered only the later stages of kernel development.

The diastatic activity of frosted wheat appears to have been rather neglected, the only reference which has come to our attention being that of Johnson and Whitcomb (12) who found the content of reducing sugar in flours after autodigestion had increased more in those from immature frozen wheat than in those from normal wheat. It is well known, however, that the gassing power of doughs from frosted wheat flour is higher than that of doughs from sound flour.

Ash. It seems to be generally agreed that the percentage of ash in wheat kernels decreases as the grain develops (5, 12, 13, 25, 29, 30, 34). Brenchley and Hall (5), Thatcher (29, 30) and Woodman and Engledow (34) found the weight of ash per kernel increases during this period, though obviously not as rapidly as the total weight of dry matter. The results obtained by Sharp (25) and Johnson and Whitcomb (12) with frozen wheat indicate that the percentage is not affected by freezing.

Experimental Material

The 1930 samples, of which the production and freezing treatment were described in the first paper of this series (18), were used for the main body of analyses reported in the present paper. These samples had been collected on 27 days during the period August 5 to September 18.

To get additional information on the normal course of ripening in wheat, for comparison with the results found with frosted material, the following subsidiary series of 19 samples was collected from the same plot of Marquis wheat in 1930. On July 21, the day on which the heads on most of the central culms were in full bloom, 5,000 heads in as nearly as possible the same condition were marked with small tags. Sampling was begun on July 26 and continued, generally at intervals of two or three days, until September 17, or about two weeks after the wheat was ripe. Collections were made at 8:30 a.m., the heads being cut from the culms, put into a covered can and taken to the laboratory. Approximately 300 heads were collected at the earlier samplings, this number being gradually decreased to 150 as the weight of the grain increased. One-third of the heads were placed at once in an oven with a rapid circulation of air at 60° C. where they were dried for 12 hr. From the remainder, the kernels were promptly removed, half of them being used for analysis in the fresh state, and the other half spread thinly on plates and allowed to dry at room temperature.

For convenience, these 19 subsidiary samples will be referred to as the "Maturity Series," to distinguish them from the main, or "Frosted Series."

Analytical Methods

Dry matter. The dry matter content of fresh kernels, flour samples, and washed gluten was determined by drying the material in a vacuum oven at 98° C. for 48 hr. In dried grain it was determined by drying a 2-gm. sample of ground material in an air oven at 130° C. for 1 hr. Grinding was usually done successively in a Wiley mill and an Arcade mill, a combination which reduced the material to a state of fineness in which it could also be used satisfactorily for nitrogen extractions, more rapidly than could be done with the Wiley mill alone.

Total nitrogen. All total nitrogen determinations were made by the Kjeldahl method, using mercuric oxide as the oxidizing agent and a two-hour digestion period.

Ammonia nitrogen. A 20-gm. sample of fresh kernels was ground as finely as possible with sand and 20 to 50 cc. distilled water in a mortar, then transferred to a distilling flask with enough distilled water to make the volume up to 250 cc. and the ammonia determined by the aeration method used by Plimmer and Rosedale (21) for ammonia in hydrolysates. This aeration method was also applied to dried wheat, after grinding in a mill, but in such material no ammonia could be demonstrated.

Salt-soluble nitrogen. A 10-gm. sample of fresh kernels was ground with sand and approximately 20 cc. of 5% potassium sulphate in a mortar, then transferred to a 250-cc. centrifuge bottle with sufficient 5% potassium sulphate to make the total volume up to 200 cc. The bottle was shaken mechanically for half an hour, then the contents was centrifuged. A 50-cc. aliquot of the clear, supernatant liquid was pipetted into a Kjeldahl flask and used for nitrogen determination. For dried, ground wheat and flour the procedure was modified by using 8 gm. of dry matter and shaking with 200 cc. of 5% potassium sulphate for 1 hr.

Non-protein nitrogen. A 100-cc. aliquot of the supernatant liquid from the above salt extraction was pipetted, with continual shaking, into a flask containing 100 cc. of 5.0% trichloracetic acid. The flask was allowed to stand for half an hour, then the contents was filtered. A 100-cc. portion of the clear filtrate was used for a Kjeldahl nitrogen determination. A discussion of the use of trichloracetic acid as a precipitating reagent will be found in a recent paper (16).

Washed gluten. Gluten was washed from flour samples using tap water which had a pH of approximately 7.5 and a salt concentration of 0.02%. The weight of flour used and the time and rate of washing were those recommended by Dill and Alsberg (9). The phosphate solution recommended by these workers was compared with the tap water, and although the weight of wet gluten obtained was slightly higher when the phosphate solution was used, there were no appreciable differences in the weights of the dry glutens.

Sugars and diastatic activity. The diastatic activity of flour samples was determined by Malloch's modification of Rumsey's method (14). The blank in this determination gives the quantity of reducing sugars present in the flour. An aliquot of the blank was inverted by the citric acid method of Davis and Daish (7) and used for the determination of invert sugar.

Ash. Ground samples of 2 gm. were ashed in a muffle furnace at 570° C. for 6 hr.

Maturity Series

The results with the maturity series are summarized in five tables and five figures, in which the samples are characterized by the number of days from flowering, given in Table I and in four of the figures, and by the dry matter content of the kernels at the time of cutting, given in all the tables.

FRESH AND DRY WEIGHTS

The progressive development of the kernels is shown in Table I by the changes in the percentage dry matter and in the actual fresh and dry weights of a thousand kernels and their original moisture content. The two sets of dry weights refer to the kernels removed from the heads immediately and *(a)* oven-dried directly, *(b)* air-dried at room temperature, then ground, resampled and oven-dried. The weights of the fresh kernels and of those

TABLE I

FRESH AND DRY WEIGHT OF WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING

Sample No.	Date of cutting	No. of days from flowering	Dry matter at cutting, %	Weight per 1,000 kernels, gm.			Weight of water per 1,000 fresh kernels, gm.
				Fresh*	Oven-dried directly*	Air-dried then oven-dried	
1	July 26	5	26.8	—	—	2.91	—
2	28	7	26.9	—	—	—	—
3	30	9	26.8	22.98	6.16	5.20	16.82
4	Aug. 1	11	26.0	28.22	7.36	6.90	22.66
5	4	14	32.0	36.63	11.72	10.45	26.18
6	6	16	35.7	41.71	14.89	13.27	28.44
7	8	18	40.2	43.98	17.68	16.24	27.74
8	11	21	44.6	46.70	20.83	19.97	26.73
9	13	23	48.9	49.35	24.15	23.43	25.20
10	15	25	50.2	53.48	26.85	25.67	26.62
11	19	29	53.2	55.68	29.63	29.31	26.05
12	22	32	55.7	56.53	31.47	31.31	25.06
13	25	35	58.4	54.33	31.71	32.00	22.62
14	28	38	59.8	54.23	32.41	31.98	21.82
15	30	40	70.4	48.22	33.94	32.44	14.28
16	Sept. 2	43	74.3	43.40	32.26	32.38	11.14
17	5	46	85.3	39.45	33.64	32.22	5.81
18	10	51	82.4	41.04	33.82	—	7.22
19	17	58	85.6	38.88	33.30	—	5.58

**Weights of Samples 3 to 8 calculated.*

oven-dried directly were, in the case of samples 3 to 8, not actually determined, but calculated from the nitrogen contents shown in Table II. The only assumption made was that the weight of nitrogen would not change during air-drying at room temperature in spite of the loss of dry matter by the respiratory oxidation of carbon.

The first four samples were collected while the kernels were still forming, and were small, thin and watery, with a dry matter content of 26 to 27%. In Samples 5 to 7, representing the dry matter range of 32 to 40%, the kernels gained rapidly in plumpness, but the watery condition and original green color persisted. In Samples 8 to 11, dry matter range 44.6 to 53.2%, the kernels changed from watery through milky to a fairly firm dough. The color changed from green through yellowish to a decided pink. These kernels, on drying slowly, acquired a slightly greenish, amber color. Sample 12, cut at 55.7% dry matter, was made up entirely of pink kernels, plump and firm, drying to a clear amber. This was the first sample to develop a tenacious gluten pad when the fresh kernels were ground with water in a mortar. Samples 13 to 17, dry matter range 58.4 to 85.3%, exhibited at the time of collection a gradual change from firm to very hard and from pink to a bright clear amber; all were clear amber when dried. By ordinary visual observation, the wheat was judged fully ripe for binder harvesting at Sample 16, dry matter 74.3%, and the dry weights of the kernels show them to have been fully developed some days earlier. Samples 18 and 19, which were allowed to stand uncut for one and two weeks after the wheat was fully ripe, were very hard, but had lost the clear brightness of the amber color which characterized the two immediately preceding.

Fig. 1 shows the percentage dry matter and weights per 1,000 kernels in the successive cuttings up to Sample 17, cut on September 5, 46 days after flowering, the samples being numbered correspondingly on the two kinds of curves. The rate of increase in dry weight accelerated somewhat in the first few samples, then was quite constant up to Sample 10, cut on August 15 at a dry matter content of 50.2%, after which it fell off gradually to maturity. The curve for percentage dry matter is divided into four approximately straight portions. The first four samples came within the period of kernel formation, during which the percentage remained at 26 to 27. The samples from 4 to 9 fall on the second straight portion, marking a period of rapid gain up to 48.9%. From Samples 9 to 14 (59.8% dry matter) the gain is much less rapid, the result of a period of dull, cool, showery weather, aided no doubt by the diminution in the rate of increase in dry weight shown in the other curves. At Sample 14, the percentage curve again begins a steeper ascent, attributable in part to the return of bright, warm weather, but perhaps still more to the onset of rapid desiccation which marks the completion of kernel development. This last part of the curve can probably also be considered straight, the irregularities being more suggestive of sampling errors than of any change in direction. Samples 18 and 19, which were allowed to stand uncut for some time after maturity, are not represented in Fig. 1, but it may be noted (Table I) that Sample 18, which was cut on a damp morning, was 3% lower in dry matter than Samples 17 and 19, which were cut on warm, bright mornings. It may be assumed that with steady weather the percentage curve, after the kernels are fully formed, would approximate more nearly to one straight line than appears in Fig. 1.

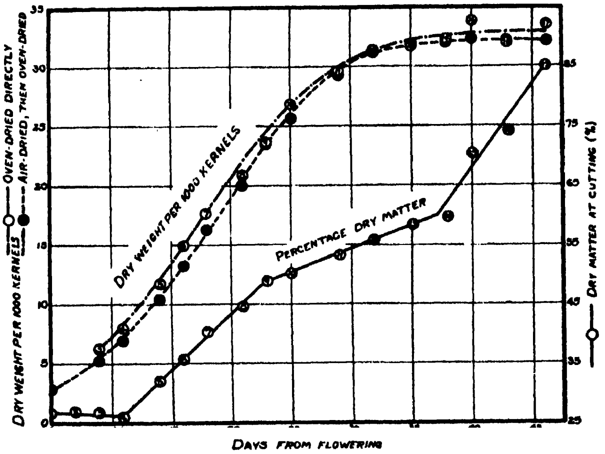

FIG. 1. *Percentage of dry matter in kernels and dry weight per 1,000 kernels at progressive stages of maturity.*

The weight of water per 1,000 kernels, shown in the last column of Table I, was fairly constant from the time the kernels were fully formed until they had reached about 56% dry matter, or a period of 18 days, which is the same as that found by Brenchley and Hall (5) at Rothamsted, as compared with 10 days found by Woodman and Engledow (34) at Cambridge. The end of this period marks the attainment of maximum fresh weight by the kernels, and further gains in dry weight were very small. There now ensued a process of rapid desiccation, which is associated with a sharp diminution in the rate, or even the complete cessation, of various physiological processes.

A comparison of the two dry weight curves, which run nearly parallel close together on Fig. 1, affords a certain rough estimate of respiration losses during the curing of grain. The upper curve represents the samples oven-dried directly after removal from the freshly collected heads. The other curve represents those allowed to air-dry in the laboratory before oven-drying. The dry weight of the latter was consistently lower up to Samples 12 and 13. Later irregularities are probably due mainly to sampling errors. The parallelism of the curves below this point suggests that, under laboratory conditions at least, the weight losses due to respiration while air-drying are fairly constant in samples containing less than 56 to 58% dry matter at cutting, though corresponding percentage losses of course become progressively less with increasing weight of the kernels. The constancy in weight losses probably means that the effect of increasing size in the developing embryo was during this period just offset by the diminishing rate of respiration consequent upon the declining percentage moisture content. At later stages the moisture factor became predominant, and the net amount of respiration then declined.

The dry weight per 1,000 kernels, determined by either method, changed very little after the grain reached a dry matter content of 56 to 58%. From this we may conclude that grain cut any time after the kernels have become pink and the dough firm, until full ripeness or even for some considerable time thereafter, will, in the absence of such interfering factors as storms, lodging, etc., give practically the same yield.

TOTAL NITROGEN

The total nitrogen content at progressive stages is shown in Table II as both percentage and weight for the kernels removed from the heads immediately on collection and *(a)* oven-dried directly, *(b)* air-dried at room temperature, then ground, re-sampled and oven-dried; and as percentage only for fresh kernels and for kernels dried in the heads at 60° C. before threshing, grinding and finally oven-drying like the others. In Fig. 2 the percentages found by the three methods of drying, and in the topmost curve of Fig. 3 the weights in the air-dried kernels, are shown graphically.

The percentage total nitrogen in fresh kernels remained about constant while the kernels were forming, then rose gradually but constantly until maturity. In the kernels oven-dried directly, there is perhaps a slight drop in this percentage shown by a comparison of Samples 1 to 3 with Samples 5

TABLE II

TOTAL NITROGEN IN WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING AND METHOD OF DRYING

Sample		In fresh kernels, %	In kernels oven-dried directly		In kernels air-dried then oven-dried		In kernels dried at 60° C. for 12 hr. then oven-dried, %
No.	Dry matter at cutting, %		%	Gm. per 1,000	%	Gm. per 1,000	
1	26.8	0.67	2.51	—	—	—	2.57
2	26.9	0.67	2.50	—	—	—	2.52
3	26.8	0.68	2.54	—	3.01	0.156	2.56
4	26.0	0.73	2.80	—	2.98	0.206	2.58
5	32.0	0.79	2.47	—	2.77	0.289	2.55
6	35.7	0.88	2.46	—	2.76	0.366	2.60
7	40.2	1.00	2.48	—	2.70	0.438	2.54
8	44.6	1.13	2.54	—	2.65	0.529	2.56
9	48.9	1.22	2.50	0.604	2.64	0.618	2.61
10	50.2	1.31	2.61	0.701	2.69	0.690	2.65
11	53.2	1.40	2.63	0.779	2.76	0.809	2.75
12	55.7	1.58	2.83	0.890	2.98	0.933	2.87
13	58.4	1.68	2.88	0.913	3.00	0.960	2.99
14	59.8	1.73	2.89	0.937	3.02	0.966	2.96
15	70.4	2.07	2.94	0.998	2.98	0.967	2.98
16	74.3	2.13	2.87	0.926	2.96	0.958	3.00
17	85.3	2.52	2.95	0.992	3.01	0.969	2.97
18	82.4	2.53	3.07	1.038	—	—	—
19	85.6	2.58	3.02	1.006	—	—	—

to 7 (the value for Sample 4 appears to be in error, and is omitted from Fig. 2). Such a small drop in early stages may be readily assigned to the cause suggested by Brenchley and Hall (5), that the pericarp tissues formed first are richer in nitrogen than the subsequent filling. It is, however, even smaller than the early decrease found by Woodman and Engledow (34) and much smaller than that reported by most workers. From this point onwards there was an almost continuous gain until maturity.

In the kernels air-dried at room temperature before oven-drying, the course of the nitrogen percentages suggests that the large initial decrease found by many workers, and here clearly seen in Fig. 2, is due largely to the method of analysis employed. Samples 3 and 4 (the first two on this particular curve) are as high as the mature samples, and at all stages except maturity the percentage nitrogen is higher than in the samples oven-dried directly. We have seen already (Table I and Fig. 1) that there was a loss of dry weight by respiration during air-drying. The loss of carbon would account for the gain in percentage nitrogen. Since the dry weight losses due to air-drying were fairly constant during the whole period of rapid gain in dry weight (Fig. 1), they would of course increase the percentage nitrogen most in the earliest (lightest) samples, and progressively less as the kernels became heavier. There is reason to believe that if the highest and lowest curve in Fig. 2 could be freed of the influence of experimental error, they would converge with mathematical regularity from kernel formation to maturity. Any initial drop which may be attributed to changes in the bran-endosperm ratio would affect them both equally, and obviously cannot have played more than a minor part in determining the slope of this part of the curve for air-dried kernels.

In the kernels dried rapidly in the heads at 60° C. the total nitrogen percentages, as may be seen in Fig. 2, occupy an intermediate position between those of the kernels dried by the other two methods. This can be readily understood, since respiration losses would be at a minimum under the very rapid drying conditions at 98° C. *in vacuo*, at a maximum under the conditions of slow air-drying at room temperature, and intermediate in a current of air at 60° C. The last-named conditions would give rise initially to intense respiratory activity, but this could not be long sustained because of the rapid loss of moisture.

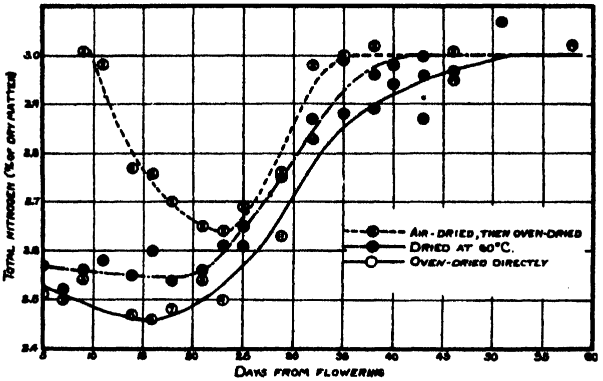

FIG. 2. *Percentage of nitrogen in dry matter of kernels dried in various ways at progressive stages of maturity.*

The material oven-dried directly undoubtedly represents most nearly the actual course of nitrogen percentage in the corresponding wheat standing in the field. The rise which succeeded the initial decline is in agreement with most modern work, and may be attributed largely to respiratory losses of carbon. The magnitude of the rise, over 0.5% nitrogen or about 3% crude protein, is greater than any we have seen reported. However, it will be remembered that McGinnis and Taylor (17) calculated that the loss of carbohydrates during a 16-day ripening period was sufficient to raise the protein 1.46%. That rate sustained over the period of about 30 days represented by the rising portion of the lowest curve in Fig. 2 would account for nearly all of the rise found in these experiments.

The topmost curve in Fig. 2, on the other hand, represents more closely the course of the percentage nitrogen in wheat cut prematurely and allowed to cure in the field. From the practical point of view it appears that whether wheat is cut or allowed to stand uncut after it reaches about 58% dry matter, the total nitrogen percentage attains approximately the same level.

Turning now to the weight of nitrogen per 1,000 kernels (Table II and top curve of Fig. 3), attention is directed chiefly to the figures for air-dried kernels, for which the series of determinations is more complete, and probably more reliable, since respiration should not affect the weight of nitrogen, and the determination was made with dried, well ground and carefully sampled material, as compared with a small quantity of arbitrarily chosen, fresh moist kernels in the case of those oven-dried directly. The agreement between the two methods, where they can be compared in Samples 9 to 17, must be considered fair, considering the difficulties of sampling. The results with the air-dried kernels show a steady increase in weight up to Sample 13, cut 35 days after flowering. There is possibly a slight further gain in Sample 14, cut 3 days later, but it seems safe to say that translocation of nitrogenous matter ceased when the wheat reached a dry matter content of 58 to 60%, a point approximating to that already seen to mark the culmination of various important processes, and for this reason considered critical. The final weight of nitrogen was about 1 gm. in 1,000 kernels.

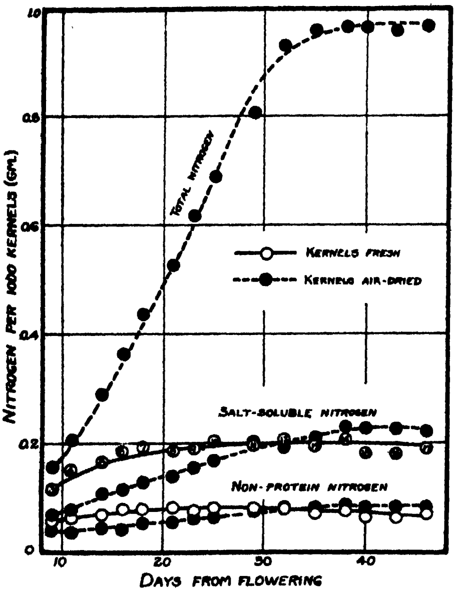

FIG. 3. *Weight of total, salt-soluble, and non-protein nitrogen per 1,000 kernels at progressive stages of maturity.*

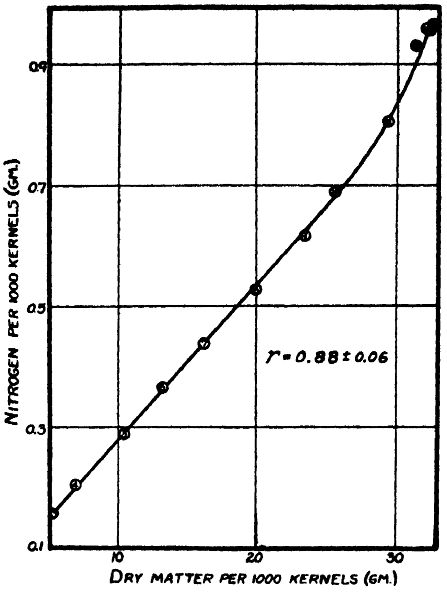

FIG. 4. *Weight of total nitrogen in relation to weight of dry matter in kernels.*

In Fig. 4, the weights of nitrogen and dry matter in the air-dried kernels are plotted one against the other. From the resulting curve it appears that the ratio of nitrogenous to non-nitrogenous substance in the material moved into the endosperm (assuming a constant fraction of this material was used in the kernel by respiration) was constant during the main period of development. Possibly it was constant during the entire period, as the upward turn at the end may have been due to a differential change in the rates of gain of total dry substance by translocation and of loss of carbohydrates by respiration, the former diminishing more rapidly than the latter as the moisture content declined.

AMMONIA NITROGEN

The analytical results for ammonia nitrogen in the fresh kernels are summarized in Table III, expressed both as percentage of total nitrogen and as weight per 1,000 kernels. From a consideration of the method of analysis and the fact that no measurable amount could be demonstrated in dried kernels, it is obvious that this fraction is free and, from the small and changing amounts present, that it is transitory in nature. The trend observed is a resultant of the relative rates of translocation into, and of synthesis within, the kernels, the latter process being no doubt conditioned largely by the amount of free water present at various stages. Ammonia received by translocation accumulates in the very watery contents of kernels in process of formation. Both percentage and weight increase until the dry matter content of the kernels reaches 32%. The percentage of ammonia nitrogen then begins to decrease—owing either to its utilization or to a disproportionate inflow of other nitrogen fractions—but the actual weight continues to increase up to 40% dry matter, indicating that the rate of utilization has not yet overtaken the rate of translocation. From then on, utilization exceeds translocation, and both percentage and weight of ammonia nitrogen decrease until at 58% dry matter only an insignificant amount is demonstrable. This is the point at which, as we have already shown, translocation of nitrogenous substances to the kernel practically ceases.

TABLE III

AMMONIA NITROGEN IN FRESH WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING

Sample		Ammonia N as % of total N	Ammonia N as weight, per 1,000 kernels mg.
No.	Dry matter at cutting, %		
1	26.8	0.67	
2	26.9	0.61	
3	26.8	1.18	1.84
4	26.0	1.28	2.63
5	32.0	1.34	3.87
6	35.7	1.18	4.32
7	40.2	1.09	4.77
	44.6	0.87	4.60
9	48.9	0.60	3.71
10	50.2	0.56	3.86
11	53.2	0.30	2.43
12	55.7	0.25	2.33
13	58.4	0.04	0.38

It is interesting to note that the quantities of ammonia nitrogen found in these samples, ranging from about 0.4 to 4.8 mg. in 1,000 kernels, were very much less than those reported by Woodman and Engledow (34), and the definite trend seen in our samples does not appear in their results, which show almost as much ammonia in fully mature kernels as at any other stage. No doubt part of the difference at least is attributable to differences in analytical methods.

SALT-SOLUBLE NITROGEN

The results of the salt-soluble nitrogen determinations, expressed as percentage of total nitrogen and as weight per 1,000 kernels, are shown in Table IV and Figs. 3 and 5.

TABLE IV

SALT-SOLUBLE NITROGEN IN WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING AND METHOD OF DRYING

Sample		In fresh kernels		In kernels dried at room temp.		In kernels dried at 60° for 12 hr.
No.	Dry matter at cutting, %	As % of total N	As weight per 1,000 kernels, gm.	As % of total N	As weight per 1,000 kernels, gm.	As % of total N
1	26.8	74.1	—	—	—	57.6
2	26.9	72.0	—	—	—	57.9
3	26.8	74.0	0.116	44.5	0.070	51.2
4	26.0	68.6	0.153	39.3	0.081	42.6
5	32.0	58.3	0.169	37.2	0.108	35.3
6	35.7	51.2	0.188	31.2	0.114	31.2
7	40.2	44.3	0.194	29.6	0.130	23.6
8	44.6	35.6	0.187	26.4	0.140	21.9
9	48.9	32.0	0.193	25.4	0.157	19.2
10	50.2	28.7	0.204	24.2	0.167	20.0
11	53.2	25.1	0.196	25.4	0.205	21.1
12	55.7	23.3	0.208	20.8	0.194	18.8
13	58.4	21.5	0.197	22.0	0.211	18.7
14	59.8	22.0	0.207	23.9	0.230	18.9
15	70.4	18.3	0.183	23.5	0.227	22.2
16	74.3	19.7	0.181	23.6	0.226	20.7
17	85.3	19.7	0.195	22.8	0.222	22.6
18	82.4	19.9	0.206	—	—	—
19	85.6	23.2	0.233	—	—	—

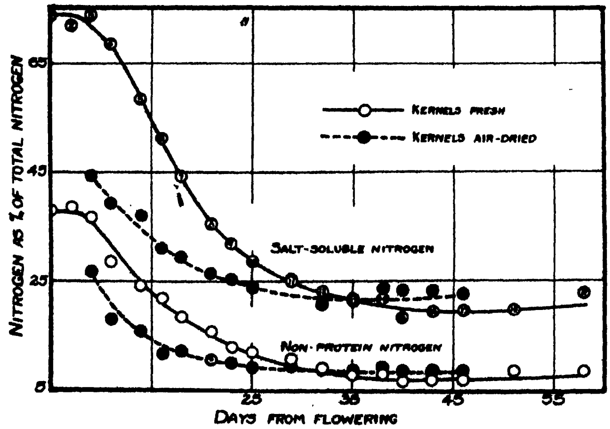

FIG. 5. *Percentages of salt-soluble and non-protein nitrogen in fresh and air-dried kernels at progressive stages of maturity.*

The data for the fresh kernels, which begin with Sample 1, indicate very little change in percentage salt-soluble nitrogen during the early formative stage, but after Sample 3 there was a decided, continuous drop until Sample

13, cut at about 58% dry matter content. Later irregularities in this percentage are probably due mainly to the difficulties of analysis; the grain at this stage was too moist to grind in a mill and yet too hard to grind effectively in a mortar, and it was difficult to ensure complete extraction of soluble nitrogen.

The air-dried kernels showed initially a very much lower percentage of salt-soluble nitrogen than the fresh kernels, owing presumably to the utilization, during the process of drying, of the fraction available for the synthesis of the gluten proteins, and no doubt in part also to a decrease in the solubility of the albumins and globulins on drying. The difference diminished as the grain progressed towards maturity, until at about 58% dry matter (Sample 13) the curves in Fig. 5 intersect. Synthesis and other changes affecting solubility apparently stopped at this point. The fact that the curve for air-dried samples ends on a slightly higher level is no doubt attributable to the difficulty of extracting the fresh kernels already mentioned, which did not obtain in the case of dried kernels. It may be concluded, therefore, that in the mature kernels about 22 to 23% of the nitrogen was salt-soluble.

The grain dried rapidly at 60° C. at first fell into an intermediate position with respect to percentage of salt-soluble nitrogen, then dropped below both the fresh and air-dried kernels. It will be remembered that in percentage of total nitrogen the kernels dried at 60° C. occupied an intermediate position throughout the whole series of samples (Fig. 2). There, however, the comparison was with kernels dried at 98° C. on the one hand and air-dried kernels on the other. Here (Table IV) the comparison is with fresh and air-dried kernels. While it is not clear why the position of the 60° kernels should have changed from intermediate to lowest in early stages, it must be set down as the resultant of processes affected by temperature: synthesis and possibly some denaturation would reduce solubility on the one hand, and hydrolysis increase it on the other. All three classes of kernels represented in Table IV reached approximately the same level in samples cut at 58 to 60% dry matter.

The weight of salt-soluble nitrogen determined in the kernels (Table IV and Fig. 3) increased slowly until about Sample 13, cut at 58% dry matter. Before this, the weight was greater in the fresh than in the air-dried kernels, owing to synthesis or changes in solubility in the latter as already suggested, but afterwards it was slightly lower in the fresh kernels, owing to the difficulty of extraction. The absence of any substantial accumulation of salt-soluble nitrogen in the kernels indicates that it is utilized in the building of higher compounds almost as rapidly as it is produced by synthesis or introduced by translocation. Of about 0.22 gm. salt-soluble nitrogen in 1,000 mature kernels, the figures for non-protein nitrogen given in the next section make it appear that rather less than two-thirds was protein.

NON-PROTEIN NITROGEN

The analytical data for non-protein nitrogen are presented in Table V in three ways: as percentage of the total nitrogen, as percentage of the salt-soluble nitrogen (of which fraction it formed a part), and as weight per 1,000 kernels. The first and third of these appear also in Figs. 5 and 3 respectively.

TABLE V

NON-PROTEIN NITROGEN IN WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING AND METHOD OF DRYING

Sample		In fresh kernels			In kernels dried at room temp.			In kernels dried at 60° C. for 12 hr.
No.	Dry matter at cutting, %	As % of total N	As % of salt-sol. N	As weight per 1,000 kernels, gm.	As % of total N	As % of salt-sol. N	As weight per 1,000 kernels, gm.	As % of total N
1	26.8	38.2	51.5	—	—	—	—	50.6
2	26.9	38.8	53.9	—	—	—	—	51.6
3	26.8	36.6	49.5	0.057	26.6	59.8	0.042	46.9
4	26.0	28.6	39.1	0.064	18.1	46.1	0.037	37.2
5	32.0	24.3	41.7	0.070	15.9	42.7	0.046	30.6
6	35.7	22.0	43.0	0.080	11.6	37.2	0.042	22.3
7	40.2	18.5	41.8	0.081	12.2	41.2	0.054	20.5
8	44.6	15.8	44.4	0.083	10.6	40.2	0.056	17.2
9	48.9	13.0	40.6	0.077	10.2	40.2	0.063	13.8
10	50.2	12.1	42.2	0.083	9.3	38.4	0.064	13.6
11	53.2	10.9	43.4	0.083	9.4	37.0	0.076	11.6
12	55.7	9.2	39.5	0.082	9.1	43.8	0.084	10.5
13	58.4	8.0	37.2	0.073	8.7	39.5	0.083	9.7
14	59.8	8.1	36.8	0.078	9.3	38.9	0.090	9.5
15	70.4	6.5	35.5	0.064	8.7	37.0	0.084	8.7
16	74.3	6.9	35.0	0.064	8.8	37.3	0.084	8.0
17	85.3	7.1	36.0	0.071	8.6	37.7	0.084	8.8
18	82.4	8.8	44.2	0.088	—	—	—	—
19	85.6	8.5	36.6	0.086	—	—	—	—

The curves, though necessarily falling lower on the scale than those for salt-soluble nitrogen in the fresh and air-dried kernels, are not unlike the latter and doubtless yield to the same explanations. The 60° kernels (Table V only) are definitely higher than the fresh and air-dried kernels until the samples contained 58 to 60% dry matter at cutting. Again we must suppose the heat to have been the cause of the difference, whether by direct hydrolysis, or by accelerated enzyme activity at 60° C.

Expressed as a percentage of the salt-soluble nitrogen, the non-protein fraction decreased gradually with the development of the kernels, indicating progressively more complete utilization in the synthesis of protein.

In all three classes of kernels, the non-protein nitrogen, which started highest in the 60° kernels, next in the fresh, and lowest in the air-dried, reached by descending gradients varying in steepness the same final level in samples containing over 58 to 60% dry matter, of about 8 to 9% of the total nitrogen, about 37% of the salt-soluble nitrogen, or about 0.08 gm. in 1,000 kernels.

Frosted Series

The 27 cuttings of Marquis wheat at progressive stages of development in 1930, subjected in all but the last case to the freezing treatment described (18), gave a series of 79 samples of grain for analysis. The analytical data reported include the nitrogen fractions and ash content of the grain, and the nitrogen fractions, washed gluten, sugar content and diastatic activity of the flour. Yield per acre of grain and nitrogen, and some calculations of translocation from straw to grain after cutting, are also reported. In all the tables presented, except Table VI, the data are arranged so that horizontal comparisons show frost effects, and vertical comparisons the effect of progressive development. As usual, the stage of development is indicated by the moisture content of the kernels at the time of cutting. Those samples marked with an asterisk were cut at the north end of the field, where the crop was later and less even in maturity, as a result of soil-blowing in the spring.

YIELD PER ACRE OF GRAIN AND NITROGEN

The yield per acre of grain and of its nitrogen content, for all check (unfrozen) samples collected at the south end of the field, where the stand was for the most part quite uniform, are presented in Table VI. The yields from the first ten cuttings were measured directly, but those from the last six cuttings were calculated, because the portion of the block from which they were collected was somewhat uneven and broken. The ratio of the yield per acre in bushels to the weight per 1,000 kernels in grams was about 0.9 in the earliest samples and increased to about 1.1 in Samples 8, 9 and 10. The latter figure was used to calculate the yields from the kernel weights of subsequent samples.

TABLE VI

YIELD OF GRAIN AND NITROGEN PER ACRE FROM CHECK SAMPLES CUT AT SOUTH END OF FIELD

Dry matter at cutting, %	Yield of grain per acre, bu.	Total N in grain, %	Yield of N per acre, lb.
31.2	11.9	3.04	21.7
34.0	12.2	2.98	21.8
34.9	14.7	3.00	26.7
38.1	15.5	3.00	27.9
39.4	19.9	3.11	37.1
40.9	18.9	2.86	32.4
43.9	23.8	2.74	39.1
45.5	26.5	2.76	43.9
46.8	28.1	2.72	45.8
46.7	32.5	2.81	54.8
54.9	36.2	2.87	62.3
56.8	39.2	2.98	70.1
57.3	40.4	2.95	71.5
58.5	40.0	3.02	72.5
65.6	41.0	3.04	74.9
69.4	40.3	3.02	73.0

The kernel weight (yield) reached an approximately constant value by the time the grain contained 57% dry matter, thus agreeing with the result for the maturity series. The yield of nitrogen per acre appears to increase slightly until a later date, but not enough to contradict the conclusion from the maturity series that translocation to the kernels ceases when these reach a dry matter content not greater than 58 to 60%. The practical conclusion from both series is that wheat cut any time between this stage and full ripeness gives about the same yield.

ASH IN GRAIN

The percentage ash in the grain, reported in Table VII, decreased gradually as the kernels developed, the final value being only about half the initial value, in the maturity range studied. Changes in cuttings more mature than

TABLE VII

ASH IN WHEAT GRAIN IN RELATION TO MATURITY AT CUTTING AND EXPOSURE TO FROST

Dry matter at cutting, %	As % of grain (13.5% moist.)			As weight per 1,000 kernels, gm.		
	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	2.56	2.18	2.29	0.346	0.296	0.294
34.9	1.94	1.95	2.11	0.322	0.354	0.358
39.4	1.82	1.88	1.77	0.359	0.386	0.346
43.9	1.58	1.44	1.50	0.369	0.350	0.357
46.8	1.43	1.43	1.51	0.374	0.367	0.391
48.3*	1.45	1.52	1.53	0.346	0.402	0.402
50.5*	1.45	1.40	1.38	0.384	0.405	0.392
51.1*	1.47	—	1.43	0.396	—	0.423
51.3*	1.39	1.39	1.43	0.406	0.390	0.388
55.8*	1.43	1.38	1.37	0.451	0.443	0.448
56.8	1.31	1.35	1.31	0.463	0.485	0.462
57.3	1.33	1.30	1.30	0.501	0.468	0.474
58.1*	1.26	—	1.26	0.442	—	0.447
59.1*	1.29	—	1.37	0.466	—	0.487
65.5*	1.26	—	1.28	0.453	—	0.425
65.6	1.21	—	1.20	0.447	—	0.444
	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	2.08	2.06	2.10	0.302	0.295	0.326
38.1	1.64	1.90	1.94	0.276	0.321	0.359
40.9	1.54	1.76	1.54	0.326	0.373	0.342
45.5	1.54	1.54	1.50	0.364	0.373	0.381
46.7	1.47	1.49	1.41	0.391	0.406	0.380
50.5*	1.38	1.42	1.42	0.383	0.354	0.374
51.1*	1.44	—	1.43	0.387	—	0.423
53.3*	1.40	1.41	—	0.413	0.397	—
54.9	1.31	1.31	1.49	0.451	0.433	0.486
57.4*	1.35	1.33	1.30	0.447	0.451	0.421
58.1*	1.31	—	1.26	0.457	—	0.447
58.5	1.35	1.32	1.38	0.494	0.480	0.497
59.1*	1.35	—	1.37	0.482	—	0.487
65.5*	1.27	—	1.28	0.468	—	0.425
65.6	1.18	—	1.20	0.421	—	0.443
69.4	—	—	1.18	—	—	0.428

**Samples from north end of field.*

are represented by 57–58% dry matter must be regarded as fortuitous, since other constituents became approximately stable in quantity at that stage, and the stooks were protected from rains which might have caused leaching. There are no differences between frozen and check samples which can be attributed to the freezing treatment. These findings are in harmony with hose cited from the literature on an earlier page.

The decreasing ash percentages during the developmental period covered y the data recorded mean of course that during an earlier period minerals were translocated relatively more rapidly than other constituents, and in this later period more slowly. This may be attributed in part at least to the early laying down of the envelope of the kernel, which is rich in ash. Over a maturity range comparable to that represented in Table VII, it can be seen in Tables I and II that the dry matter and nitrogen content of air-dried kernels both trebled in quantity, whereas the weight of ash (Table VII) increased perhaps 50%.

NITROGEN FRACTIONS IN GRAIN

The total nitrogen, salt-soluble nitrogen, and non-protein nitrogen in the grain, as affected by stage of maturity at cutting and exposure to 4, 8, 10 and 14 degrees of frost, are shown in Table VIII. To save space, these data

TABLE VIII

NITROGEN FRACTIONS IN WHEAT GRAIN IN RELATION TO MATURITY AT CUTTING AND EXPOSURE TO FROST

Dry matter at cutting, %	Total nitrogen, %			Salt-soluble N as % of total N			Non-protein N as % of total N		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	2.94	3.16	3.04	34.7	30.0	30.9	14.2	10.8	10.5
34.9	2.94	3.12	3.00	29.3	27.2	28.7	12.3	10.3	10.3
39.4	2.94	3.00	3.11	28.2	26.0	26.0	12.3	10.3	10.0
43.9	2.68	2.74	2.74	25.4	24.1	25.5	11.6	10.2	10.2
46.8	2.78	2.78	2.72	25.9	25.5	24.3	10.4	10.1	9.2
48.3*	2.60	2.72	2.72	25.0	25.7	25.7	11.5	10.3	9.9
50.5*	2.57	2.58	2.67	24.9	23.3	24.0	11.7	10.5	9.4
51.1*	2.52	—	2.52	24.6	—	25.4	11.1	—	9.5
51.3*	2.55	2.63	2.54	23.5	23.6	25.2	10.6	9.9	9.4
55.8*	2.67	2.68	2.60	25.5	24.6	25.8	9.0	9.0	8.5
56.8	2.84	3.01	2.98	22.5	21.9	21.8	8.5	7.3	7.4
57.3	2.84	2.85	2.95	22.2	21.8	21.7	7.7	7.7	7.5
58.1*	2.76	—	2.76	23.9	—	23.6	9.1	—	8.0
59.1*	2.74	—	2.66	24.1	—	24.8	9.5	—	9.4
65.5*	2.78	—	2.70	23.7	—	25.9	9.4	—	8.9
65.6	3.06	—	3.04	22.9	—	23.4	9.2	—	8.2
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	2.70	2.78	2.98	34.8	35.3	29.5	21.5	18.7	10.4
38.1	2.72	2.70	3.00	30.9	30.4	26.7	18.4	17.0	10.3
40.9	2.66	2.66	2.86	27.4	27.8	25.1	15.0	14.3	9.8
45.5	2.63	2.65	2.76	25.9	25.8	23.9	11.8	11.3	9.1
46.7	2.72	2.71	2.81	26.5	25.1	24.9	11.0	10.7	9.2
50.5*	2.56	2.56	2.65	24.6	23.8	23.4	12.1	10.9	9.8
51.1*	2.47	—	2.52	24.3	—	25.4	11.1	—	9.5
53.3*	2.59	2.58	2.63	25.5	23.3	23.6	12.4	11.6	9.5
54.9	2.80	2.78	2.87	22.1	21.6	21.6	8.6	8.6	7.7
57.4*	2.63	2.65	2.69	24.3	24.1	24.5	9.9	9.3	8.2
58.1*	2.76	—	2.76	23.9	—	23.6	9.1	—	8.0
58.5	3.05	3.00	3.02	21.6	22.0	21.9	7.9	8.0	7.3
59.1*	2.73	—	2.66	24.2	—	24.8	9.5	—	9.4
65.5*	2.78	—	2.70	23.7	—	25.9	9.4	—	8.9
65.6	3.06	—	3.04	22.9	—	23.4	9.2	—	8.2
69.4	—	—	3.02	—	—	—	—	—	—

**Samples from north end of the field.*

are presented only on a percentage basis, since most of the points brought out by the weights per 1,000 kernels have already been discussed adequately in connection with the maturity series. The weights of total nitrogen in some of the samples are, however, used in a later table in calculating translocation and respiration.

The check (unfrozen) samples in this frosted series correspond quite closely to the air-dried samples of the maturity series, the chief differences being that the latter came from heads plucked by hand, rubbed out and air-dried in the laboratory, while the former came from binder-cut swaths, air-dried in the field in stooks protected from weathering. It is observable in Table VIII that in the early cuttings (up to about 48% dry matter) the check samples are slightly higher in per cent total nitrogen than the corresponding air-dried samples in the maturity series (Table II, column 6), and lower than these in salt-soluble nitrogen (Table IV, column 5) and in non-protein nitrogen (Table V, column 6). This means that, as might be expected, drying was slower in the stook than in the laboratory, that consequently the period of physiological activity was prolonged, leading to greater losses of carbon by respiration, thus raising the per cent total nitrogen, and to more complete synthesis, thus reducing the per cent salt-soluble and non-protein nitrogen. In later cuttings the values for corresponding samples in both maturity and frost series are approximately equal. The conclusions from the maturity series are thus fully sustained by the results from the check samples in the frosted series.

Exposures to four degrees of frost had no apparent effect on the per cent total nitrogen or on the salt-soluble and non-protein fractions. Exposures to 8, 10, or 14 degrees of frost, in the earlier cuttings, reduced the per cent total nitrogen and raised the percentages of the fractions. The effect on the total nitrogen can be explained if we assume that frost injury to the germs checks respiration during subsequent drying and thus leads to a higher ratio of carbon to nitrogen in the dried kernels. The same frost injury would check synthesis, and result in the higher percentages observed in the salt-soluble and non-protein fractions. The stage of maturity at which these frost effects ceased to be evident varied with the severity of exposure and was not the same for all quantities measured. Slight differences in non-protein nitrogen appeared to persist to full maturity, but no differences in total or salt-soluble nitrogen, even after the most severe exposures, were found in samples cut with a dry matter content of more than about 57%.

NITROGEN FRACTIONS IN FLOUR

The total nitrogen, salt-soluble, and non-protein nitrogen of the flour milled from the grain samples just described, are given in Table IX, and show almost precisely the same relations to maturity at cutting and frost exposures. No differences in either grain or flour were found in the wheat exposed to four degrees of frost, while the differences resulting from heavier frost characterized both grain and flour alike.

TABLE IX

NITROGEN FRACTIONS IN FLOUR MILLED FROM FROZEN AND CHECK SAMPLES OF WHEAT

Dry matter at cutting, %	Total nitrogen, %			Salt-soluble N as % of total N			Non-protein N as % of total N		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	2.76	3.08	2.97	21.0	18.8	19.9	8.7	6.2	6.1
34.9	2.74	2.86	2.88	20.1	17.5	18.1	7.7	5.6	6.2
39.4	2.90	2.94	3.10	17.2	14.6	15.5	6.2	4.8	5.2
43.9	2.59	2.58	2.70	18.5	15.5	14.8	6.6	5.0	4.4
46.8	2.68	2.74	2.74	16.4	15.3	15.7	6.0	5.5	5.1
48.3*	2.46	2.60	2.60	17.1	17.3	18.1	6.9	6.5	6.5
50.5*	2.42	2.44	2.58	16.5	15.2	14.7	7.4	5.9	5.4
51.1*	2.34	—	2.48	17.9	—	16.1	7.7	—	6.0
51.3*	2.47	2.41	2.40	17.4	16.6	16.7	7.7	6.6	6.2
55.8*	2.70	2.63	2.68	15.6	15.6	15.2	7.4	6.1	5.2
56.8	2.68	2.82	2.93	13.4	12.8	13.3	5.2	4.3	4.8
57.3	2.63	2.67	2.67	14.4	15.7	14.2	5.0	5.2	4.5
58.1*	2.50	—	2.74	12.8	—	14.9	4.8	—	5.1
59.1*	2.58	—	2.50	14.5	—	15.2	6.4	—	5.6
65.5*	2.52	—	2.52	16.7	—	16.7	5.6	—	5.2
65.6	2.83	—	2.84	15.1	—	14.4	4.6	—	4.2
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	2.56	2.60	2.82	27.3	26.5	18.4	16.4	13.5	6.0
38.1	2.56	2.56	2.84	25.3	22.7	17.3	14.1	12.5	6.3
40.9	2.56	2.50	2.73	19.9	19.6	15.4	10.2	8.4	5.1
45.5	2.52	2.46	2.64	17.5	16.7	15.2	7.5	6.5	5.3
46.7	2.60	2.63	2.74	15.4	14.8	17.5	6.5	6.8	5.5
50.5*	2.45	2.42	2.52	18.8	17.4	17.5	8.6	7.4	6.3
51.1*	2.40	—	2.48	18.3	—	16.1	9.2	—	6.0
53.3*	2.49	2.51	2.46	20.4	18.3	17.9	9.6	8.0	6.5
54.9	2.48	2.50	2.46	17.7	16.4	17.5	6.5	5.6	5.7
57.4*	2.46	2.49	2.46	14.6	12.0	13.8	6.3	5.2	4.9
58.1*	2.50	—	2.74	12.4	—	14.9	5.2	—	5.1
58.5	2.82	2.79	2.82	11.3	11.5	11.7	5.0	5.0	4.6
59.1*	2.58	—	2.50	15.1	—	15.2	6.2	—	5.6
65.5*	2.42	—	2.52	16.9	—	16.7	6.2	—	5.2
65.6	2.83	—	2.84	15.5	—	14.4	5.3	—	4.2
69.4	—	—	2.84	—	—	14.8	—	—	4.2

**Samples from north end of the field.*

The fact that no chemical differences could be detected between lightly frozen and check samples, even though the former suffered external changes and a lowering of grade (18), while heavy frost caused in immature samples chemical changes which persisted in the flour, possibly explains some of the conflicting statements in the literature reviewed earlier in this paper. Investigators would be led to different conclusions, depending upon the stage of maturity and severity of exposure of their experimental material.

WASHED GLUTEN

The results of the washed gluten determinations are given in Table X. The modification of Dill and Alsberg's method already described was used for all flours except those from wheat of the first four cuttings exposed to 10 and 14 degrees of frost. To avoid complete dispersion of the dough during washing, it was necessary in these cases to use Olson's method (19) of blending the samples with equal parts of a strong flour. While by this method substantial quantities of "gluten" were recovered, it may be doubted that the material was entitled to such a classification, since by itself it possessed none

TABLE X

WASHED GLUTEN OF FLOUR MILLED FROM FROZEN AND CHECK SAMPLES OF WHEAT

Dry matter at cutting, %	Dry weight as % of flour			Texture and tenacity			Color			Gluten / Flour protein**		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	16.0	15.8	15.9	1	3	6	1.5	1.5	2	1.02	0.90	0.94
34.9	14.6	17.6	17.2	2	4	6	1.5	2	2.5	0.94	1.08	1.05
39.4	16.0	19.6	17.5	3	4	6.5	2	2	2.5	0.97	1.17	0.99
43.8	14.7	16.1	17.5	2	4	7	2	2.5	3	0.99	1.10	1.14
46.8	16.0	16.4	16.2	3	5	7.5	2	2.5	3	1.05	1.05	1.04
48.3*	14.9	16.8	17.0	3	4	7	1.5	2	2.5	1.06	1.14	1.15
50.5*	14.0	14.1	16.3	4	5	7.5	2.5	3.5	4	1.01	1.01	1.11
51.1*	13.4	—	15.0	5	—	7.5	2.5	—	4	1.01	—	1.06
51.3*	14.2	15.0	14.4	5	7	8	2.5	3.5	4	1.01	1.09	1.05
55.8*	17.2	16.3	17.0	6	7	8	3	4	4.5	1.12	1.09	1.11
56.8	15.1	18.8	18.9	6.5	8.5	9.5	3	4	5	0.99	1.17	1.13
57.3	17.6	19.6	19.6	7	9	10	4	5	5	1.17	1.29	1.29
58.1*	17.6	—	16.2	8	—	9	4.5	—	5	1.24	—	1.04
59.1*	16.7	—	16.0	9	—	9	5	—	5	1.14	—	1.13
65.5*	17.4	—	16.3	10	—	10	5	—	5	1.21	—	1.13
65.6	17.5	—	17.5	10	—	10	5	—	5	1.09	—	1.08
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	10.8	—	17.8	0	—	6	0	—	2	0.74	—	1.16
38.1	13.3	12.0	17.6	0	0	7	0	0	2.5	0.91	0.82	1.09
40.9	16.0	14.4	17.3	0	0	7	0	0	3	1.10	1.01	1.11
45.5	18.1	18.3	15.5	0	0	7.5	0	0	2.5	1.26	1.31	1.03
46.7	15.6	14.6	18.0	1	0.5	7.5	0	0	3	1.05	0.97	1.15
50.5*	15.3	12.9	16.4	1	2	7	1	1	3	1.09	0.93	1.14
51.1*	13.6	—	15.0	2	—	7.5	1	—	4	0.99	—	1.06
53.3*	15.6	14.0	16.8	3	5	8	2	3	4	1.10	0.98	1.20
54.9	16.4	17.9	19.6	5	6	9	2	3	4	1.16	1.26	1.40
57.4*	16.6	15.6	16.6	5	7	8.5	4	4	4.5	1.19	1.10	1.19
58.1*	16.0	—	16.2	7.5	—	9	3.5	—	5	1.13	—	1.04
58.5	17.9	18.2	19.0	8	9	10	4	4.5	5	1.11	1.14	1.18
59.1*	15.0	—	16.0	8	—	9	4	—	5	1.02	—	1.13
65.5*	16.5	—	16.3	9	—	10	5	—	5	1.20	—	1.13
65.6	17.3	—	17.5	9.5	—	10	5	—	5	1.07	—	1.08
69.4	—	—	17.8	—	—	10	—	—	5	—	—	1.10

**Samples from north end of field.*
***Protein = total nitrogen × 5.7.*

of the physical characteristics of gluten. At best, the quantities in the first three cuttings were well below those recovered from the corresponding checks without the necessity of blending.

The physical properties of the gluten were difficult to score, because some samples which became slack while being washed yielded a firm tenacious gluten when freed from starch, while others remained slack. The final product from samples which slackened during washing was always spongy in texture, with coarse vesiculation. Texture and tenacity were scored 0 in gluten from flours which could be washed only when blended; 5 when the gluten was firm and tenacious but coarse in texture; 10 when the gluten was firm, tenacious and fine textured; and intermediately for intermediate conditions. Color scores ranged from 0 to 5, 0 being given to the very dark, grey gluten from severely frozen immature samples, and 5 to the creamy gluten from mature material.

The tendency of poor gluten to disperse readily into particles of various sizes was demonstrated by washing the dough of immature, froze nsamples over screens of various sizes, from which the loose material caught could be collected from time to time. For one sample the results were as follows:

Screen	Yield, %	Texture	Color
30 mesh	1.2	1	4.5
64 mesh	6.8	1	2.5
12XX bolting cloth	14.6	0.5	0

The texture of the product did not vary much, but the color of the fraction most resistant to dispersion was very much better than that of the rest. The bolting cloth used as a routine part of the procedure in washing all samples could usually be omitted with little if any loss, but was quite necessary to secure a satisfactory yield of these samples which became very slack during washing.

The yields of dry gluten obtained from the check samples, while they show considerable variation which must be attributed to experimental errors of various kinds, were on the whole about the same at all stages. Exposure to four degrees of frost had no apparent effect. The effect of heavier frost on the gluten yields of the very immature samples was masked of course by the blending procedure used. Even so, the yield in most of these cases was less than that of the corresponding check or 4° samples. The ratio of dry gluten to the crude protein content of the flour, shown in the last three columns of the table, was in all but a few cases, again chiefly among the very immature, heavily frozen samples, a little greater than unity.

The lowest score for texture and tenacity awarded any check gluten was 6, and there was gradual improvement from the earliest cuttings to those made at about 57–58% dry matter. The admixture of green kernels in the grain samples from the north end of the field is reflected in the lower quality of their gluten, as compared with that of the more uniform samples from the south end cut at corresponding dry matter contents. Immaturity had a greater effect upon color than upon texture and tenacity, as seen in the steeper gradient of the color scores of the checks, from the earliest cuttings to those made at 57–58% dry matter.

It will be seen that even four degrees of frost damaged the gluten quality, color being affected up to the maturity represented by 56.8% dry matter, and texture and tenacity up to 57.3% dry matter, the last cutting exposed to this amount of frost. Heavier frost increased the amount of the injury and extended the range of susceptibility, which included, with respect to texture and tenacity, even the most mature samples subjected to 14 degrees of frost.

It must be concluded that the physical properties of the gluten are more easily affected by frost than any chemical property studied in the grain or flour. This of course is very important from the point of view of baking quality, a subject to be considered in the next paper.

Sugar Content and Diastatic Activity of Flour

The sugar content and diastatic activity of the flours milled from the frozen and check samples of wheat are given in Table XI. Diastatic activity is indicated by the maltose production of 10 gm. of flour digested for one hour under the conditions of the method already cited.

The reducing sugar made up little more than 10% of the total sugar. Both reducing and invert sugar percentages declined in the earlier check samples, but not so much as found by Brenchley and Hall (5) and Thatcher (30), who analyzed grain brought directly from the field and dried promptly in the laboratory. It has been shown earlier in this paper that respiration during slow drying, such as would take place in these samples cured in stooks, was sufficient to change appreciably the composition of the kernels, the greatest change occurring in the most immature samples (Fig. 2). No doubt much of the free sugar is used up in the process.

Four degrees of frost was insufficient to cause any consistent differences in the sugar content, but 8, 10 and 14 degrees led to increasing amounts of reducing sugar in flours from grain cut while containing less than 57–58% dry matter. There are no differences in the percentages of invert sugar which can be attributed to the freezing treatment of the grain.

If we make the assumption suggested when discussing the total nitrogen content of the frozen grain, that frost injury to the germs slows up respiration during subsequent drying, then we have at least one possible explanation why the frozen samples are higher in reducing sugar than the check samples, and why the differences from the checks increase with immaturity of the grain and severity of frost exposure. The laboratory germination percentages (to be reported elsewhere) of the grain samples from which these flours were milled, were reduced in the two most extreme cases from 100 and 99 in the checks to 9 and 16 in the frozen samples; and even these figures do not tell the whole story, as field tests showed the seedling vigor in the frozen samples

TABLE XI

SUGAR CONTENT AND DIASTATIC ACTIVITY (MALTOSE PRODUCTION) OF FLOUR MILLED FROM FROZEN AND CHECK SAMPLES OF WHEAT

Dry matter at cutting, %	Reducing sugar %			Invert sugar %			Maltose produced per hour, mg.		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	1.29	0.92	0.94	6.56	6.09	—	245.5	212.0	—
34.9	0.96	0.59	0.69	5.33	5.23	5.51	253.8	186.8	160.8
39.4	0.92	0.67	0.58	4.28	4.42	4.42	197.7	136.1	132.2
43.9	0.72	0.63	0.52	3.57	3.98	4.04	279.7	202.6	154.0
46.8	0.51	0.50	0.52	3.60	3.81	3.75	239.2	185.2	180.1
48.3*	0.70	0.74	0.72	3.96	3.90	3.92	270.0	198.8	181.7
50.5*	1.01	0.69	0.98	4.05	3.68	3.98	316.8	227.2	151.9
51.1*	0.82	—	0.55	3.78	—	3.19	226.0	—	168.0
51.3*	0.78	0.53	0.62	3.44	3.90	3.81	218.6	239.4	221.5
55.8*	0.52	0.48	0.45	3.25	3.04	3.50	423.9	340.7	174.9
56.8	0.66	0.64	0.58	3.51	3.90	4.12	233.8	142.9	122.4
57.3	0.55	0.44	0.46	3.60	3.66	3.90	154.4	132.4	135.3
58.1*	0.53	—	0.51	3.20	—	3.52	137.9	—	154.4
59.1*	0.53	—	0.51	3.00	—	3.28	193.2	—	119.0
65.5*	0.57	—	0.54	3.36	—	3.34	105.4	—	157.4
65.6	0.51	—	0.48	3.35	—	3.38	148.0	—	97.2
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	—	—	0.94	—	—	6.05	—	—	155.3
38.1	2.16	1.85	0.61	4.14	3.39	5.21	443.9	434.2	131.6
40.9	1.11	1.04	0.59	3.80	3.65	3.57	453.2	503.0	179.7
45.5	0.91	0.65	0.63	3.74	3.44	3.64	419.7	189.7	145.8
46.7	0.71	0.66	0.54	3.40	2.88	3.46	472.4	243.0	165.8
50.5*	1.22	0.89	0.76	3.81	3.40	3.80	280.8	316.7	187.4
51.1*	1.15	—	0.55	4.30	—	3.19	348.6	—	168.0
53.3*	0.91	0.74	0.49	3.52	3.01	3.28	385.4	348.5	199.6
54.9	0.82	0.57	0.45	3.42	3.42	3.50	241.8	226.4	171.3
57.4*	0.53	0.46	0.49	3.19	2.85	3.46	245.4	250.4	161.2
58.1*	0.55	—	0.51	3.31	—	3.52	219.0	—	154.4
58.5	0.49	0.52	0.51	3.19	3.53	3.59	151.2	113.8	105.4
59.1*	0.56	—	0.51	3.24	—	3.28	205.8	—	119.0
65.5*	0.53	—	0.54	3.29	—	3.34	145.9	—	157.4
65.6	0.49	—	0.48	3.25	—	3.38	166.7	—	97.2
69.4	—	—	0.45	—	—	3.42	—	—	122.7

**Samples from north end of field.*

to have been very much impaired. If we assume respiration to be a characteristic of living cells and to vary with vital activity, the other assumption would seem to follow.

On the other hand, Bailey and Gurjar (2) reported the respiratory activity of frosted wheat to be greater than that of sound wheat. Their conclusion, however, is based on "two samples of commercial wheat containing frosted kernels." Since samples of this description frequently originate in fields caught by night frost while immature, it seems possible that immaturity rather than frost explained their results. In any event, some of the data obtained in the present study accord better with the hypothesis that frost injury retards respiration, and pending a more critical experiment there does not seem sufficient warrant to reject it.

Bailey and Gurjar's suggestion that the disorganization of the protoplasm by frost sets free hydrolytic enzymes and leads to an accumulation of split products, such as dextrose, may be readily accepted. Our own figures for diastatic activity, recorded in the last three columns of Table XI, though erratic, show in general that this property declined with maturity and that frost increased it decidedly, even four degrees having a noticeable effect, and heavier exposures proportionately more. The frost effects appeared to persist to full maturity, though they were of relatively minor importance in samples cut at 58% dry matter or later.

Part of the reducing sugar accumulation noted in the flour samples from wheats frozen while immature may reasonably be attributed, therefore, to accelerated enzyme activity in the kernels after thawing. This explanation is, however, inapplicable to the effect of frost on the per cent total nitrogen in the grain, noted earlier.

TRANSLOCATION AND RESPIRATION AFTER CUTTING

The higher weights of nitrogen and dry matter per 1,000 kernels in the checks as compared with the heavily frozen samples, among those cut while immature, suggested that translocation from the vegetative parts to the kernels must have taken place in the former after cutting and stooking. In the left-hand section of Table XII, these values are given for the first seven

TABLE XII

TRANSLOCATION AND RESPIRATION AFTER CUTTING IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST

Dry matter at cutting, %	Actual data						Calculated data				
	Weight per 1,000 kernels, gm.			Weight of protein per 1,000 kernels, gm.			Theoretical weight[a] per 1,000 kernels, gm.		Respiration losses[b] per 1,000 kernels, gm.		Gross gains[c] of checks by translocation, gm.
	Frozen	Check	Net gain by translocation	Frozen	Check	Net gain by translocation	Frozen	Check	Frozen	Check	
34.0	12.48	13.41	0.93	1.949	2.280	0.331	13.9	16.3	1.4	2.9	3.8
38.1	14.60	16.00	1.40	2.252	2.736	0.484	15.9	19.3	1.3	3.3	4.7
40.9	18.33	19.20	0.87	2.776	3.129	0.353	19.5	22.0	1.2	2.8	3.7
45.5	20.71	21.97	1.26	3.112	3.454	0.342	21.8	24.1	1.1	2.2	3.5
46.7	23.28	23.34	0.06	3.602	3.739	0.137	25.0	26.0	1.7	2.6	2.7
50.5*	22.78	22.78	0.00	3.329	3.443	0.114	22.6	23.4	−0.2	0.6	0.6
53.3*	24.95	24.82	−0.13	3.676	3.722	0.046	24.5	24.8	−0.4	0.0	−0.1

**Samples from north end of field.*

[a] *Calculated from actual weights of protein in frozen and check samples, and percentages of protein in samples of maturity series oven-dried directly.*

[b] *Differences between actual and theoretical weights per 1,000 kernels.*

[c] *Sums of actual net gains in weight per 1,000 kernels by translocation and of calculated losses by respiration.*

cuttings exposed to 10 and 14 degrees of frost, averaged for the two frozen samples in each cutting, and with the nitrogen expressed as crude protein (N × 5.7). The apparent net gains of the checks by translocation, being the differences between the figures for frozen and check samples given in columns 4 and 7, are quite substantial in the first four cuttings, after which they diminish rapidly to negligible proportions. To the extent that translocation was not stopped by the frost, these differences would be proportionately too small, but it will be shown below that translocation after freezing was probably negligible.

On the other hand, it is quite clear from the nitrogen percentage relations in the earlier cuttings that respiration occurred in both frozen and check samples after stooking. The nitrogen percentages in the heavily frozen samples are of the order of those found in the maturity series in samples air-dried in the laboratory, while those of the checks and lightly frozen ones are still higher. The lowest percentages of nitrogen were found of course in the samples oven-dried directly. These differences appear to be explainable only on the basis of different amounts of carbon loss by respiration after cutting, following naturally from the different rates of drying these various samples, modified in certain cases by the freezing treatments.

To get some idea of the order of these respiration losses, the calculations reported in the right-hand side of Table XII were resorted to. If we accept the percentages of nitrogen found in the maturity series in the samples oven-dried directly, as representing the true ratio of protein to dry matter at a given stage of maturity, and assume that the departures from this ratio in other samples cut at corresponding stages are due to respiration after cutting, then the elimination of this respiration would lead to the theoretical weights per 1,000 kernels indicated in columns 8 and 9, from which can be deduced the respiration losses given in the next two columns. Calculated this way, the loss from the frozen samples was about half that from the check samples.

The theoretical weights of the frozen samples are of about the same order as the actual weights of the samples in the maturity series cut at the same stages of maturity (found by graphic interpolation of the values in Table I) and oven-dried directly. The first four may be compared as follows:

Frozen	13.9	15.9	19.5	21.8
Dried directly	13.4	16.2	18.3	21.7

It may be concluded, therefore, that the freezing treatment, while it reduced respiration only to about half the intensity found in the checks, reduced translocation to negligible proportions. We are justified then in the case of the checks to add to their actual net gain by translocation the amount of their loss by respiration, to get their gross gain by translocation after cutting, as given in the last column of the table.

Respiration of course continues in the fully matured grain as long as it remains alive, but the method employed here, subject as it is to sampling and other experimental errors and depending upon assumptions which can be regarded only as approximations, is too rough to show consistent losses except in the more immature samples. The negative quantities occurring among the values for the last two samples in the table are evidences of the errors involved, though it is less surprising to find them attached to samples from the north end of the field, which have given somewhat erratic results throughout all these experiments, and are moreover not strictly comparable with samples of the maturity series, all of which came from the south end. The data appear to justify the conclusion that respiration losses after cutting are of substantial size in grain cut at a dry matter content of less than 50%.

Translocation after cutting must stop earlier than respiration, but all we can conclude from these results is that it probably is substantial only in samples with less than 50% dry matter at cutting. This relation of maturity to translocation after cutting is in harmony with the results of the joint experiment by Saunders (23) and Shutt (27), cited at the outset of this paper. The failure of Arny and Sun (1), Wilson and Raleigh (33) and Greaney (10) to detect in the weights of kernels cut at different stages of maturity any evidence of translocation after cutting immaturely, may be laid at least in part to their failure to allow for respiration losses, which under the conditions of the present experiment appear to offset about two-thirds of the gain by translocation.

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THE MICRO-ORGANISMS IN PROFILES OF CERTAIN VIRGIN SOILS IN MANITOBA[1]

By M. I. Timonin[2]

Abstract

Twelve profiles of five different kinds of virgin soils of Manitoba were sampled, described and critically examined for soil organisms, hydrogen ion concentration and the moisture and organic matter present. Usually the A horizon showed the highest count of each group of micro-organisms and the C horizon the lowest, although the greatest number of bacteria were present in the B horizon of Soil 1 in the month of May. The proportion of anaerobic bacteria and fungi to total numbers increased with the depth of the horizon. Moisture content of the soil was not found to exert any consistent effect upon the numbers of micro-organisms present. Fungi were most abundant in the wooded and peat soils, bacteria more so in soils of the meadow-prairie phase. In the wooded soils the microbiological horizons appeared to coincide with the morphological horizons.

Introduction

The physical and chemical features of various horizons in typical soil profiles have been considered by many investigators, but little information in relation to the soil organisms is on record.

This work represents a continuation of a previous study (4) on the identity, prevalence and significance of fungi found in Manitoba surface soils. It covers detailed investigation into the micro-organisms, moisture, organic matter and pH reaction of the various horizons in 12 profiles representing five distinct virgin soils of Manitoba.

Brown and Benton (7) made microbiological studies of some typical Iowa soil profiles. They report the largest number of bacteria and actinomycetes in the A_1 or A_2 and the largest number of fungi at times in the A_3 sub-horizons. The most striking decreases occurred from the A_3 to the B sub-horizons, although further decreases in each successive sub-horizon were noted. Fungi decreased in numbers relatively less than actinomycetes, and actinomycetes less than bacteria. Variation in moisture content seemed to have no definite relation to the number of micro-organisms, especially in lower horizons. A seasonal effect on numbers was noted although this was confined chiefly to the A_1, A_2 and A_3 sub-horizons. In some cases soil type differences were reflected by the number of micro-organisms in the various horizons. Organic matter, as noted by color, was observed to be the one significant characteristic influencing quantitative occurrence of organisms. Little relation existed between variations in conditions in the subsoil and counts of micro-organisms, although more bacteria and actinomycetes were found in the subsoils of loess type than in those of drift origin.

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This and part of the succeeding paper formed a thesis submitted to the Committee on Graduate Studies, University of Manitoba, in partial fulfilment of the requirements for the degree of Master of Science.

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McKibbin and Gray (13), studying chemical and microbiological factors in Quebec soils, noted that microbiological activity is dependent to some extent on the amount of water present in the soil. They considered that soil type has a greater influence on the numbers of micro-organisms than season. Later Gray and McMaster (11), in a study of podsol soils, submitted evidence in support of the view that numbers of bacteria as well as microbiological activity, as measured by carbon dioxide production and ammonification of urea, appear to be influenced by organic matter relations of well differentiated horizons.

Newton (14) reported that the highest bacterial count was obtained in Alberta soils in the spring. Waksman and Purvis (18) determined the numbers of micro-organisms in Highmoor and Lowmoor peat profiles in New Jersey. In their conclusions they noted that the sphagnum layer of Highmoor soils contains relatively few bacteria, but below the sphagnum layer, or as soon as the sedimentary layers of the peat are reached, there is a great increase in the number of bacteria. In the case of fungi a rapid decrease in numbers with increasing depth was noted.

Several workers have reported on micro-organisms at different depths in the soil, without correlating depth and soil horizon.

Methods of Sampling and Analyzing

A pit was dug in a representative area of each soil and blocks of soil about 8 in. (20 cm.) square were cut from the vertical side, showing characteristic horizons. The successive layers of the profile thus obtained were wrapped in heavy paper for transportation to the laboratory. Field notes recording the depth of the various horizons and their characteristics, temperature of each horizon, root development and surface flora were taken. In the laboratory a portion was removed from each horizon for moisture determination and the remainder of the block was rewrapped and held in a cool basement until the following day, when microbiological analyses were made on a moisture-free basis, using standard bacteriological technique for diluting and counting (5, 10).

The percentage of soil organic matter was determined by Tiurin's volumetric method, using Ischerekoff's coefficient for organic matter (17). The method was modified slightly: 25 ml. of 0.2 *N* chromic acid was used instead of 50 ml. After boiling, the solution was made up to 250 ml. with distilled water. One hundred ml. of the latter was titrated against Mohre's solution. The results were multiplied by 2.5 and the percentage expressed on the moisture free basis.

The hydrogen ion concentration was measured by a Leeds and Northrup potentiometer, using a quinhydrone electrode with a saturated calomel cell (2, 3). The moisture equivalent was determined by the method outlined by Briggs and McLane (6) and the hygroscopic and wilting coefficients calculated by Alway and Russel's method (1).

The soils reported on in this investigation are classified and described according to Nikiforoff's scheme of soil classification which was modified by Ellis for Manitoba soil surveys (9). They represent five different virgin soils.

Soil I

Profile 1

Profile 1 was taken from the meadow-prairie phase of the Fort Garry association in the Red River Valley combination in the chernozem zone. The area sampled is located at 49° 48′ north latitude and 97° 10′ west longitude, about three miles west of the Pembina highway and two miles south of the city of Winnipeg. The parent material is alluvium and shallow lake deposit laid over the deep laminated lacustrian clay of Lake Agassiz.

The topography of the area is level and the precipitation water collects in the low-lying areas. The micro-relief of the region is hummocky, the hummocks being 3–5 ft. in diameter and 4–6 in. high. The pit for sampling was dug through the diameter of the hummock and the profile samples were obtained below the centre of the hummock.

Ellis and Shafer (8) have reported on the prevailing climatic conditions in this area in relation to soil phenomena, presenting the monthly rainfall and maximum, minimum and mean monthly temperatures for each growing season at Winnipeg for more than 50 years previous to 1928. The averages of the mean monthly temperatures during the growing season for the 53 year period 1875–1927 were as follows: April 38.01; May 51.98; June 62.17; July 66.43; August 63.53; September 53.97; and October 41.01° Fahrenheit. The winter precipitation, principally snow, is light, and has varied in the period 1875–1927 from 1.96 inches (recorded as water) in 1921 to 8.75 inches in 1882. The mean rainfall for April to October for the period 1872 to 1927 was 16.15 inches. For the year 1933 the monthly precipitation and maximum, minimum, and mean temperatures at Winnipeg are shown in Table I.

TABLE I

Monthly precipitation and maximum, minimum, and mean temperatures at Winnipeg, 1933

Month	Temperature in deg. Fahrenheit			Precipitation in inches		
	Max.	Min.	Mean	Snow	Rain	Total
January	33.4	−30.6	2.3	10.4	—	1.04
February	39.1	−42.0	−1.9	6.1	—	0.61
March	48.4	−18.8	17.1	2.7	0.20	0.47
April	72.2	13.8	36.9	7.8	0.20	0.98
May	84.0	27.0	54.7	—	5.27	5.27
June	96.8	35.0	68.0	—	0.97	0.97
July	91.2	46.6	69.1	—	1.61	1.61
August	93.2	38.2	66.7	—	3.63	3.63
September	79.0	31.8	56.6	—	2.69	2.69
October	72.8	13.6	36.3	6.6	0.01	0.67
November	40.2	−16.2	17.5	17.2	—	1.72
December	35.0	−42.4	−4.6	15.7	—	1.57
Total				66.5	14.58	21.23

The vegetative cover consists of a solid sod composed of the following grasses, *Poa pratensis*, *P. compressa*, *Agropyron repens* and *A. tenerum* but mixed with *Grindelia squarrosa*, *Argentina anserina*, *Aster multiflorus*, species of *Antennaria* and *Solidago*. *Salix* sp., *Symphoricarpos occidentalis*, and *Populus tremuloides* dot the surface in low-lying areas.

Ants were active on some of the hummocks.

Description of Profile 1. A_1 (0–2.5 in.) consists of a well decomposed dark brown organic material mixed with black silty clay, and a well developed mat of roots. This sub-horizon can be easily separated from A_2.

A_2 (2.5–5 in.) consists of black clay in closely packed, hard, dull-faced granules.

B_1 (5–11 in.) is a silty clay, gray in color, streaked vertically with darker colors. The horizon has a weakly developed structure, being crumbly, friable and highly calcareous. Sub-horizon B_1 slowly grades into B_2; the line of demarcation is hard to discern. Roots are not as abundant as in A_2.

B_2 (11–19 in.) is a very fine sandy clay, buff colored, crumbling easily. Specks of iron concretion are common. Root proliferation is slight. Sub-horizon B_2 is hard to separate from the parent material.

C (19–31 in.) is a light buff colored clay and very fine sand, with thin layers of gray clay richly speckled with iron accumulation. Pinholes of decayed roots are quite numerous.

This profile represents normal meadow-prairie soil. The tongued intrusions at the margins of the hummocks are due to the cracking of the soil, which occurs during dry spells, and of intrusion of surface soil through these cracks into the subsoil (8).

Profile 2 is a duplicate of Profile 1, obtained 13 days later (May 15) by removing the loose earth and cutting back the face of the pit about 1 ft. before sampling.

Profile 3, taken on June 20, represents the same type of soil as Profile 1. It was located in the zone of transition of the Red River Valley association into the Fort Garry association. Islands of alkalinized Red River and Fort Garry meadow prairie soils are intermixed in this area.

The location of Profile 3 is about two miles southeast of the area of Profile 1, about 100 yards west of Pembina highway and about 300 yards northeast of the electric power line. The surface of this area is less hummocky and the hummocks are larger in diameter and lower in height. Vegetation is apparently the same as in Profile 1.

Profile 4 is a duplicate of Profile 1. The pit was dug about 25 yards northeast of Profile 1. The samples were obtained September 21.

The results of microbiological and chemical analyses of Profiles 1 to 4 are shown in Table II.

TABLE II

PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSES OF SOIL I

Profile no.	Date of sample	Depth of horizons, in.	Temperature in °C.	Total moisture, %	Moisture equivalent	Moisture ratio†	pH in H_2O	pH in KCl	Organic matter, %	Bacteria*	Actinomycetes*	Anaerobic bacteria*	Fungi*	Algae*	Protozoa*
1	May 2, 1933	A_1(0–2.5)	9	37.9	45.42	0.83	7.31	6.68	8.04	49,150,000	4,800,000	1,000,000	26,000	1,000	–
		A_2(2.5–5)	7	27.7	37.27	0.74	8.35	7.26	3.18	131,800,000	2,200,000	1,000,000	2,167	5,000	–
		B_1(5–11)	5	30.5	34.53	0.88	8.27	7.45	2.41	158,300,000	700,000	10,000,000	320	0	–
		B_2(11–19)	3	29.3	32.82	0.89	8.24	7.54	1.76	45,300,000	700.000	1,000,000	220	0	–
		C (19–31)	0	24.9	19.70	1.26	8.16	7.69	0.86	6,000,000	0	1,000	90	0	–
2	May 15, 1933	A_1(0–2.5)	17	25.6	52.39	0.49	7.71	6.81	19.44	37,830,000	2,430,000	–	28,166	10,000	–
		A_2(2.5–5)	10	27.1	39.99	0.68	7.95	6.84	5.81	124,266,000	2,000,000	–	3,000	5,000	–
		B_1(5–12.5)	6	31.6	38.60	0.82	8.11	7.24	2.36	147,066,000	600,000	–	388	500	–
		B_2(12.5–20)	3	31.1	31.94	0.97	8.07	7.38	1.22	33,380,000	620,000	–	220	40	–
		C (20–27)	0	21.3	21.14	1.01	8.01	7.75	0.77	6,033,000	0	–	40	0	–
3	June 20, 1933	A_1(0–1.5)	27	14.8	40.37	0.37	8.03	7.00	8.96	19,200,000	3,825,000	1,000,000	46,750	10,000	40
		A_2(1.5–4.5)	24	24.2	41.00	0.59	8.08	6.98	6.54	26,525,000	4,125,000	100,000	40,500	1,000	20
		B_1(4.5–8.5)	21	16.2	39.95	0.41	8.12	7.20	2.44	4,700,000	400,000	100,000	1,000	0	0
		B_2(8.5–17.5)	20	22.3	37.90	0.59	8.29	7.44	1.56	2,310,000	77,000	100,000	632	0	0
		C (17.5–38)	17	20.0	23.22	0.86	8.12	7.52	0.87	217,000	0	1,000	98	0	0
4	Sept. 21, 1933	A_1(0–2)	12	82.0	53.67	1.52	7.46	6.75	22.25	19,000,000	3,250,000	1,000,000	60,127	1,000	100
		A_2(2–6)	11	24.3	32.06	0.76	8.08	6.83	8.64	16,500,000	3,000,000	1,000,000	6,000	500	20
		B_1(6–16)	12	31.7	41.72	0.76	8.09	6.80	2.45	16,725,000	650,000	1,000,000	2,500	0	0
		B_2(16–27)	14	31.7	33.87	0.94	8.25	7.31	1.27	2,513,000	152,000	1,000	200	0	0
		C (27–44)	13	18.8	19.17	0.95	8.27	7.49	0.31	280,000	0	1,000	40	0	0

†*Moisture ratio* $= \dfrac{\textit{Total moisture}}{\textit{Moisture equivalent}}$; * = *Number of organisms per gm. of moisture-free soil;* – = *No data available.*

SOIL II

One profile, No. 5, was taken from Soil II, about 50 yards north of Profile 3. This soil differs from Soil I only in being an alkalinized phase instead of a meadow-prairie phase. The profile is characterized by a granular structure in the upper part of the poorly defined B horizon, and by the formation of columns about 7 × 3 in. in the grayish brown clay of the lower part of the B and the upper part of the C horizons (10 to 40 in. in depth). The counts of micro-organisms in Soil II were similar to those in Soil I. Details are not given in this paper, but the species of fungi isolated are dealt with in the paper immediately following this.

SOIL III

Profile 6 was taken June 29 from the wooded phase of the phytomorphic associate of the Fort Garry association in the Red River Valley combination in the chernozem zone.

The location of the profile is about six miles north of Profile 1 and about one mile west of the Assiniboine river.

The vegetation consists chiefly of poplars, *Populus tremuloides* and *P. balsamifera*, with clumps of oaks, *Quercus macrocarpa*, (up to about seven inches in diameter) and *Corylus americana*, *C. rostrata*, *Viburnum pubescens*, *Crataegus coccinea*, *Symphoricarpos occidentalis*, *Pyrola asarifolia*, *Aralia nudicaulis*, *Carex stenophylla* and *Unifolium canadense*.

Earthworms were observed frequently.

Description of Profile 6. A_0 (0–1¼ in.): leaf mat well decomposed, penetrated by mycelium and rhizomorphs of fungi.

A_1 (1¼–3½ in.): dark fine sandy clay, powdery, with buckwheat-sized granules, mixed with finely divided organic material and penetrated by numerous roots.

A_2 (3½–7 in.): whitish-gray, heavy, very fine sandy loam with aggregations of granules on which specks of silica can be seen. Roots penetrate this horizon vertically.

B (7–17 in.): coffee-brown silty clay, shading to a lighter color in the lower part of the horizon. The structure is coarsely granular, breaking into fine granules. It is quite compact with a finer texture than A_2. Root development is more profuse than in A_2.

C (17–36 in.): light buff colored clay with very fine sand. It is the same material as in horizon C of Profile 1.

This profile represents a slightly podsolized soil. The horizons are in the process of formation and are gradually assuming the features of podsol soils.

Profile 7 represents the same soil as Profile 6. It was taken about one-half mile north of Profile 6. The surface vegetation is practically the same. The samples were obtained July 10.

Profile 8 is a duplicate of Profile 6 made by removing the loose earth from the original pit and cutting back one face about a foot before sampling. The samples were taken August 28.

The results of microbiological and chemical analyses of Soil III are shown in Table III.

SOIL IV

Profile 9 was taken from a podsolic soil area developed from an alluvial deposit. It was sampled on July 19 and plated July 20.

The sampled area is located about 50° 15′ north latitude and 96° 03′ west longitude, at the southern limits of the town of Lac du Bonnet and about 300 yards from the shore of Lac du Bonnet. The average temperature is slightly lower and the precipitation slightly higher than at Winnipeg, although 1933 was exceptionally dry at Lac du Bonnet.

The topography of the region is rolling but the area where the samples were obtained is more of a plain sloping towards the lake. This area is heavily wooded and has considerable underbrush. The vegetation of this area consists principally of the following: trees, *Populus tremuloides, Betula* sp., *Fraxinus nigra, F. pennsylvanica* and a few *Pinus resinosa;* shrubs, *Amelanchier alnifolia, Rhamnus alnifolia, Cornus stolonifera, Acer spicatum, Viburnum opulus, Ribes americana, Lonicera oblongifolia, Rubus triflorus, Corylus americana, C. rostrata;* and herbs, *Aralia nudicaulis, Geum strictum, Unifolium canadense, Smilacina stellata, Mitella nuda, Aster* spp., *Lappula echinata.*

Description of Profile 9. A_0 (0–4 in.): undecomposed and partly decomposed forest debris forming a well defined mat.

A_1 (4–5 in.): very thin and poorly developed; composed of silty clay mixed with organic debris, finely granular and dark colored.

A_2 (5–8 in.): whitish-gray fine sandy loam with indistinct line of demarcation from the overlying sub-horizon A_1, but quite distinct from the underlying horizon B. It has a platy structure which crushes into an ash-like powder; no visible organic matter.

B (8–19 in.): dark olive-brown silty clay with a finely nutty structure, aggregated into larger clumps an inch or so in diameter; compact and heavier in texture than A_2. Roots are developed very well in this horizon, and a few earthworms were observed.

C (19–37 in. and deeper): alluvial deposit of clay and very fine sand, light brown with accumulation of calcium. Structure is friable and coarser in texture than horizon B.

Profile 10 is of the same soil as Profile 9 and was obtained about one half mile from the lake and about two miles south of the town. It was sampled July 19, and the wrapped samples were held in a cool basement until July 31

TABLE III

PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSES OF SOIL III

rofile no.	Date of sample	Depth of horizons, in.	Temperature in °C.	Total moisture, %	Moisture equivalent	Moisture ratio	pH in H_2O	pH in KCl	Organic matter, %	Bacteria*	Actinomycetes*	Anaerobic bacteria*	Fungi*	Anaerobic fungi*	Fungi at 37°C.*	Algae*	Protozoa*
6	June 29, 1933	A_0 (0–1.5)	18	99.0	108.69	0.91	7.39	6.86	60.78	1,000,000	122,500	100,000	117,000	1,120	450	1,000	40
		A_1 (1.5–3.5)	17	36.8	62.19	0.59	6.77	5.57	27.01	30,000,000	7,500,000	1,000,000	15,625	1,010	100	1,000	10
		A_2 (3.5–7)	15	14.4	25.97	0.55	6.36	5.36	3.52	1,835,000	85,000	1,000,000	4,250	590	40	20	0
		B (7–17)	14	23.9	44.27	0.54	7.14	5.97	2.12	4,300,000	950,000	1,000,000	995	540	0	0	0
		C (17–36)	12	18.5	22.44	0.82	8.16	7.54	0.35	205,000	4,000	10,000	120	50	0	0	0
7	July 10, 1933	A_0 (0–2.5)	20	60.6	127.86	0.47	7.41	6.92	61.34	10,650,000	4,350,000	1,000,000	224,375	330	–	1,000	40
		A_1 (2.5–3.5)	18	17.8	27.55	0.65	7.21	6.61	19.18	31,000,000	9,000,000	1,000,000	26,875	540	–	1,000	20
		A_2 (3.5–10)	16	15.2	24.39	0.62	6.67	5.53	3.44	7,150,000	1,550,000	10,000	30,125	500	–	0	0
		B (10–18)	15	21.1	27.65	0.76	7.20	5.99	1.59	10,600,000	1,800,000	1,000,000	2,450	40	–	0	0
		C (18–36)	14	19.0	33.21	0.57	8.16	7.31	0.66	389,000	19,000	10,000	110	20	–	0	0
8	August 28, 1933	A_0 (0–2)	14	83.3	75.05	1.11	7.31	6.82	40.37	12,850,000	2,700,000	1,000,000	345,000	1,380	100	5,000	100
		A_1 (2–3)	15	37.0	33.17	1.12	6.30	5.65	11.46	850,000	0	100	3,500	40	0	0	10
		A_2 (3–7.5)	15	16.9	25.55	0.66	6.50	5.82	3.13	5,195,000	1,905,000	15,300	10,000	380	10	20	0
		B (7.5–19)	15	20.7	36.81	0.56	7.30	5.96	3.38	13,500,000	2,500,000	100,000	16,400	330	0	0	0
		C (19–36)	14	12.1	13.64	0.89	7.95	7.16	0.32	710,000	60,000	1,000	550	30	0	0	0

*= *Number of organisms per gram of moisture-free soil.*

–= *No data available.*

before plating. The description of the profile is the same as that of Profile 9, except that the B horizon is more compact and the aggregations of granules are somewhat larger in size. The results are presented in Table IV.

SOIL V

Profile 11 was taken from a peat soil, about three miles west of Pinawa Falls and five miles east from the shore of Lac du Bonnet. This peat was formed in a valley about one-half mile wide where there is no outlet for drainage water.

The vegetation of the valley area consists chiefly of the following: *Larix laricina*, *Picea canadensis*, *Betula glandulosa*, *Populus tremuloides*, *Chamaedaphne calyculata*, *Andromeda polifolia*, *Oxycoccus oxycoccus*, *Ledum groenlandicum*, *Geum strictum*, *Equisetum* spp., *Carex* spp., and *Aster* spp. The plants mentioned above grow amongst *Sphagnum* moss, together with some *Polytrichum* moss.

A pit for sampling was dug down to the heavy blue clay and samples were taken at three depths.

Description of Profile 11. 1 (0–7 in.). The surface layer is a growing sphagnum layer which varies considerably in depth.

2 (7–35 in.). The second layer is a felted moss layer intermixed with branches of trees. Waterlogging begins at 20 in. and water gradually increases with the depth. The sample was taken at a depth of from 27 to 35 in.

3 (58–64 in.) Well decomposed remains of the sphagnum moss are mixed with sticks and limbs of trees, the bark of which is decomposed. An odor of hydrogen sulphide is noticeable. Below 64 in. is a heavy blue clay layer.

Profile 12 represents the same area as Profile 11. The pit was dug about 100 yards south and across the road from Profile 11. The morphological and organic characteristics are the same as in Profile 11.

The results are given in Table V.

Results of Studies

The numbers of bacteria and actinomycetes were highest in the second or third depths, except in peat and in Profile 10, where the first depth gave the highest count, and in Profile 8, where the B horizon was highest. The counts of bacteria and actinomycetes in the B_2 sub-horizons of Soil I were similar to the counts in the B horizons of Soils III and IV, being in all cases, except Profile 8, between 11.7 and 31.5% of the highest counts in the profiles. The counts in the C horizons were uniformly low.

The counts of actinomycetes ranged from 0 to 43.7% of the total number of micro-organisms counted on Brown's sodium albuminate agar (10). They were more abundant in one of the two surface sub-horizons in all profiles. There was a marked decrease in the B horizons in the prairie soils, which was

TABLE IV

PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSES OF SOIL IV

Profile no.	Date of sample	Depth of horizons, in.	Temperature in °C.	Total moisture, %	Moisture equivalent	Moisture ratio	pH in H_2O	pH in KCl	Organic matter, %	Bacteria*	Actinomycetes*	Anaerobic bacteria*	Fungi*	Anaerobic fungi*	Fungi at 37°C.*	Algae*	Protozoa*
9	July 19, 1933	A_0 (0–4)	17	56.9	132.17	0.43	6.39	6.01	91.92	16,915,000	1,335,000	1,000,000	205,000	1,326	400	500	40
		A_1 (4–5)	15	27.1	69.68	0.39	6.21	5.45	77.02	73,000,000	16,000,000	10,000,000	65,000	970	195	5,000	20
		A_2 (5–8)	14	14.1	29.65	0.48	5.94	5.17	17.90	7,650,000	950,000	10,000	7,500	1,650	0	100	0
		B (8–19)	11	21.4	40.28	0.53	7.38	6.24	17.26	9,750,000	1,250,000	100,000	14,750	2,380	0	100	0
		C (19–37)	11	19.4	30.68	0.63	7.94	7.18	0.63	463,000	97,000	10,000	1,840	680	0	0	0
10	July 19, 1933	A_0 (0–3.5)	17	48.7	122.96	0.40	6.55	5.83	64.17	28,300,000	5,700,000	100,000	242,500	2,850	105	1,000	100
		A_1 (3.5–5)	15	23.1	71.52	0.32	5.51	4.99	25.00	4,500,000	3,500,000	1,000,000	20,000	2,650	100	100	20
		A_2 (5–8)	14	13.6	29.39	0.46	5.50	4.64	4.47	1,550,000	950,000	10,000	1,625	880	20	0	0
		B (8–23)	11	19.1	33.68	0.57	6.37	5.95	2.60	3,300,000	900,000	100,000	10,625	2,640	0	0	0
		C (23–37)	11	18.7	35.74	0.52	7.81	7.06	0.95	1,142,000	120,000	10,000	1,470	1,100	0	0	0

* = *Number of organisms per gm. of moisture-free soil.*

TABLE V

PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSES OF SOIL V

Profile no.	Date of sample	Depth of layers, in.	Temperature in °C.	Total moisture, %	Moisture equivalent	Moisture ratio	pH in H_2O	pH in KCl	Organic matter, %	Bacteria*	Actinomycetes*	Anaerobic bacteria*	Fungi	Anaerobic fungi*	Fungi at 37° C.*	Algae*	Protozoa*
11	July 19, 1933	1(0–7)	17	500.7	220.95	2.27	5.35	4.19	85.75	3,600,000	1,000,000	100,000	168,300	35,800	4,050	10,000	10
		2(27–35)	12	620.2	224.37	2.76	5.47	4.93	91.55	1,050,000	200,000	100,000	1,625	470	0	500	0
		3(58–64)	9	750	251.38	2.98	5.68	5.00	98.31	48,000	0	100,000	500	70	0	20	0
12	July, 19 1933	1(0–18)	17	500.7	190.22	2.63	4.78	3.86	90.24	2,900,000	1,150,000	100,000	372,500	30,950	1,075	10,000	10
		2(30–37)	12	620.2	188.92	3.28	5.43	4.92	68.00	2,740,000	10,000	100,000	3,100	530	0	500	0
		3(70–76)	9	750	232.91	3.22	4.39	4.68	88.91	63,500	0	100,000	875	50	0	0	0

less noticeable in the wooded soils. The prairie soils showed no growth of actinomycetes from the C horizons of four profiles, while the C horizon of the wooded soils gave counts ranging from 4,000 to 120,000 per gm. of soil.

Anaerobic bacteria varied in number throughout the profiles with the C horizons generally showing low counts.

The highest counts of fungi were found in the surface sub-horizons in all cases. The decreases in the numbers of fungi were most striking in the second sub-horizons. In the lower horizons the decreases were less marked. Even in the C horizons considerable numbers of fungi were found.

The number of fungi growing at 37° C. represented from 0–1.6% of the counts on plates incubated at 20° C. Growth at the higher temperature was confined to the fungi in the A_0, A_1 and A_2 sub-horizons.

Plates incubated in a carbon dioxide chamber yielded the same genera as those under aerobic conditions, but the colonies were not as numerous, varying from 0.6 to 78.6% of the counts on the plates incubated aerobically.

The algal flora is concentrated almost entirely in the A horizon.

Protozoa were found only in the two surface horizons. In the majority of cases ciliata only were observed. Amoebae were also found, however, only in the surface horizons in Profiles 4, 8, and 10.

Discussion of Results

In a review of the literature on the subject, one finds that most workers report that organic matter, moisture, hydrogen ion concentration, season and soil type are the factors which control the density of micro-organisms in the soil.

In this report the above mentioned factors are presented in Tables II, III, IV and V. In order to trace the influence of these factors on Manitoba soils, each will be discussed under a separate heading.

Organic Matter

There is no doubt that organic matter is the most important factor in the life of the majority of micro-organisms, because it is a source of food and energy.

Analyzing the results obtained, it is evident that the highest percentage of organic matter is found in the surface horizon in all profiles studied, except peat.

Comparing the number of micro-organisms with the recorded percentage of organic matter in the corresponding horizons which are morphologically well differentiated, the data seem to confirm the view expressed by others, that organic matter is the factor which controls the activity of the soil micro-organisms during the vegetative period. The subsoil horizons contain only a small fraction of organic matter as compared with the surface horizons and also a correspondingly low fraction of the number of micro-organisms. Yet micro-organisms decrease with depth in peat, despite a high content of organic matter throughout.

In the wooded soils sub-horizon A_2, in all cases but one, showed a higher percentage of organic matter than horizon B. However, the total number of bacteria and actinomycetes in the wooded soils III and IV was higher in the B horizon than in A_2. In the case of fungi, a higher number was also recorded from horizon B than from A_2 in the wooded soils of Profiles 8, 9, and 10. In prairie soils a similar variation in the number of bacteria and actinomycetes occurred in the profiles which were sampled during May, but in the later samples the A horizon gave larger counts. Considering these facts, the conclusion may be drawn that organic matter is not the only factor influencing the microbiological population of soil horizons.

Moisture

An analysis of the data makes it evident that the total moisture content of the soils studied does not show the true picture of the soil moisture. For example, A_1 of Profile 1 contained 1.52 times as much moisture as horizon C of the same profile. However, the moisture equivalent shows that the A_1 sub-horizon can retain 45.42% of moisture against a pull of 1,000 times gravity exercised over a period of 40 min. On the other hand, horizon C retained only 19.7%. Also, the moisture ratio $\left(\frac{\text{T.M.}}{\text{M.Eq.}}\right)$ of horizon C is 1.58 times as great as that of sub-horizon A_1. That means that horizon C contains more available moisture for plant growth than A_1. For this reason it would be a mistake to take into consideration the total soil moisture as a factor influencing the microbiological population in soil horizons. The moisture ratio of the morphologically corresponding horizons of profiles obtained from the same soil at different dates shows no definite relation to the biological population of these horizons. Horizons in which the moisture ratio is below the wilting point may show higher numbers of micro-organisms than horizons which contain moisture available for plant growth.

Profile 8 of Soil III showed an abnormal distribution of micro-organisms in comparison with the other wooded soil profiles studied. The samples of Profile 8 were obtained August 28, three days after a heavy rain occurred. The rain started August 22 and continued until August 25. A total precipitation of 1.82 in. was recorded during the four days. Perhaps owing to this fact, sub-horizon A_1 gave the lowest counts of bacteria, actinomycetes and anaerobic bacteria and the second lowest of fungi in the profile. The pH was also lowest in sub-horizon A_1. The acidity of this sub-horizon was a limiting factor for bacteria and actinomycetes, but the fungi and algae should not be affected by this. However, the fungi were also much reduced in numbers. Accordingly, acidity can not be considered as the limiting factor. Possibly there is another soil factor acting as a soil disinfectant, but the data are too uncertain to warrant any conclusions.

Acidity

Thornton and Bear (15, pp. 398–400) obtained the highest counts of soil bacteria on agar at pH 7 and at slightly alkaline reactions. Russell (15, Chap. VI) states that the "general result is that fungi preponderate in acid soils, while bacteria become numerically much more important in neutral soils, but this is not because fungi thrive better in an acid than a neutral medium but because they tolerate acidity better than bacteria."

All the profiles of the wooded soils studied except Profile 8 just mentioned, showed the lowest pH in the A_2 sub-horizons. Owing probably to this fact, the numbers of bacteria and actinomycetes also were lower than in the B horizons. In prairie soils this phenomenon does not occur because all horizons are alkaline. In organic soils, Soil V in this case, the acidity of the soil is more pronounced than in the other soils studied, and the bacteria and actinomycetes lowest in numbers. From these results we may conclude that acidity is a factor lessening the number of bacteria and actinomycetes in the soil profile.

Season

A seasonal effect on the numbers of bacteria and fungi was reported by Brown and Benton (7) in Iowa soils. Conn (see 15, p. 401) claimed that freezing of soil increases the number of bacteria. Lochhead (12) found higher numbers of bacteria in frozen soil than at ordinary summer temperatures and much larger numbers of bacteria when the soil begins to thaw.

Table II shows that in Soil I the numbers of bacteria were higher in the May samples than in the June and September samples. It appears that in this soil, bacteria decrease with increasing soil temperatures. A decrease in the number of bacteria in the late season occurred in all horizons, including C. The numbers of fungi were altogether different. They were low in May samples, but increased in horizons A and B in September samples. The numbers of fungi in B_2 and C remained practically the same throughout the season. The data obtained indicate that with increasing soil temperature corresponding to the advance of the summer season, the number of bacteria decreases throughout all the horizons in the profile; a seasonal effect apparently occurs in all horizons of the soil profiles. On the contrary, the numbers of fungi nearer the surface increased as the soil temperatures increased. A seasonal effect on the fungi apparently does not occur in B_2 and C.

Soil Type

The numbers of micro-organisms obtained during June and July from the surface horizons of meadow-prairie, wooded and organic soil types, show that bacteria and actinomycetes in meadow-prairie soils are 1.6 to 20.5 times as great as in wooded and 5 to 5.7 times as great as in organic soil types. Fungi, however, are greater in number in wooded and organic than in meadow-prairie soils. The most noticeable characteristic in wooded soils is the striking increase of bacteria and actinomycetes from the A_0 to the A_1

sub-horizons, which was recorded from all profiles except 8 and 10. This exception in Profile 8 is perhaps due to excessive precipitation and in 10 is probably explained by the fact that the samples were kept for 12 days in the basement before plating. Sub-horizon A_2 in wooded soils is one from which substances are leached by percolating water and deposited in horizon B (15, Chap. IV). Owing to this fact the B horizon showed, in nearly all cases, a higher number of bacteria, actinomycetes and fungi than the A_2 horizon, even though the B horizons are heavier in texture and located deeper than the A_2 horizons. The numbers of micro-organisms in the well differentiated horizons of wooded soil profiles seem to be closely related to the morphological appearance of the horizons; in other words, in wooded soil the microbiological and morphological horizons are identical.

The numbers of protozoa obtained from virgin soils in Manitoba were very low, compared to those reported by Russell and others (15, pp. 403–410). Strelkov (16), studying degraded chernozem soils by Koffman's method, reported that no active forms were found in the surface soil. The quantitative protozoan population of the degraded chernozem soil was reported as follows: *Amoebae* 10–100 per gm., Flagellata 5,000–7,500 and Infusoria 10–100 per gm. Taking into consideration the above figures, the virgin soils of Manitoba showed a small population of protozoa.

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FUNGI ISOLATED FROM SOIL PROFILES IN MANITOBA[1]

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Abstract

Representative samples of the fungi in the soil horizons of 12 profiles of five types of virgin soil in Manitoba were identified. The isolations were made by the standard technique; and in addition to the routine method of incubating dilution plates aerobically at 25° C., other plates were incubated aerobically at 37° C., others aerobically at about 6° C., and others anaerobically at about 20° C. Fifty-six fungi not previously known in Manitoba soil were obtained. The more important species of fungi found in soil are discussed.

Introduction

In a previous paper (3) the writers reported on the fungi isolated from various surface soils in Manitoba, the majority of which were under cultivation, although a few were virgin forest or prairie. The present study deals with an investigation of the fungi in the various horizons of 12 profiles representing five different types of virgin soil. In a preceding paper, Timonin (20) presented descriptions of the soils studied, reported on the numbers of the various groups of micro-organisms found and showed the relations between numbers and types of micro-organisms and certain chemical and physical factors. This report includes the identification of the fungi isolated in this project and also a number not determined at the time the first paper (3) was presented. The list of soil fungi that follows includes only species not previously recorded from Manitoba soils.

Additional Fungi from Manitoba Soils

MYXOBACTERIALES

Myxococcus ?ruber Baur was found to be present in 24 of 32 isolations made from Profile 6. Drops of 1 : 10 dilution from sub-horizons A_1, A_2 and B_1 were placed on Ashby's mannitol phosphate agar (7). A few tests were made from other profiles and a yellow but undetermined member of the Myxobacteriales was found to be fairly common, and a pink species less common. These preliminary tests demonstrate that Myxobacteriales occur in Manitoba soils.

MYXOMYCETES

Myxomycetes occur commonly in Manitoba, and their spores fall in considerable numbers upon the ground. The few tests made with soil placed upon the mannitol phosphate agar (7), however, failed to disclose their presence.

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PHYCOMYCETES

Cunninghamella elegans Lendner. Four cultures of this striking member of the Mucorales were obtained from plates made for Myxomycetes from sub-horizon A_0 of Profile 6. The standard technique of isolation from soil failed to demonstrate the presence of this species.

Mortierella elasson Sideris and Paxton. A fungus which is this species or much like it was obtained frequently. The spores and sporangiophores are even smaller than described for *M. elasson.* The mycelium is persistently white, and in many isolations grows in the broadly zonate manner illustrated by Sideris and Paxton (18, Fig. 1). The hyphae usually are 4–6μ wide, but vary from 1 to 10μ, and are non-septate and vacuolate. Sporangiophores appear tardily or not at all on most media; on corn-meal agar they arise on the aerial mycelium, are usually simple although rarely branched, 30–100μ long and usually 4–8μ wide at the base and 2–3μ at the apex; sporangia are spherical, 8–20μ in diameter, the wall rupturing easily, leaving a slight turned down collar; the spores are oval to subspherical, mostly 2.5–4 × 3–6μ, thin walled, hyaline, with one or more guttulae; chlamydospores (gemmae) occur commonly on potato-sucrose agar and are 9–22μ in diameter. Different isolations vary somewhat but they seem to represent a single species near enough to *M. elasson* to be included with it, although possibly it deserves varietal rank. This form was found to be common in meadow soil, especially in the deeper horizons, and occasional in forest soil, alfalfa fields and elsewhere.

Mortierella vinacea Dixon-Stewart. This fungus, reported by Dixon-Stewart (6) from cultivated and bush soils in Australia, seems to be commonly present in forest and peat soils in Manitoba. The cultures usually are white at first, then often russet vinaceous as described, but there is considerable variation in the depth and shade of color in different isolations and on different media. The mycelium is irregular, mostly 4–8μ in diameter, but in older cultures bears many swellings (chlamydospores or gemmae) with guttulae. Sporangiophores may be branched or simple, and bear globose sporangia 15–50μ in diameter. Spores in sporangia are subglobose, 2–3μ in diameter, but swell greatly on the approach of germination and readily form germ tubes in water.

Mucor ?varians Povah. A few cultures, apparently of this species, were obtained from Soil IV.

Zygorhynchus vuilleminii Namys. This species was found a few times in the different horizons of Profile 7.

ASCOMYCETES

Chaetomium olivaceum Cke and Ell. (*C. globosum* Kunze). Five isolations of this fungus were obtained from surface soils.

Chaetomium setosum Wint. This species developed once in a culture from Horizon B incubated at 37° C.

Humarina ?convexula (Pers.) Seaver. A Discomycete with white to pale yellow apothecia, ascospores 12–16 × 8–10μ with two guttulae, and paraphyses thickened up to 16μ at the apex, developed in five cultures from Soil III incubated at 37° C.

?Pleospora sp. Two cultures from Soil IV produced perithecia and muriform spores.

Sclerotinia sp. One isolation from a surface soil was considered by Prof. Whetzel to be probably the haploid state of some *Sclerotinia.*

Xylaria sp. Three cultures from the surface of Soil I, incubated at 37° C. then transferred to flasks of leaves etc., developed striking *Xylaria*-like fruit bodies, but asci were not found.

FUNGI IMPERFECTI

Acremoniella sp. A few cultures were obtained which produced dark terminal spores (chlamydospores?). These might be considered to belong to *Acremoniella* as considered by Saccardo, but their exact status is uncertain.

Acrostalagmus albus Preuss var. ***varius*** Jensen. Three cultures from Soil III.

Aspergillus nidulans (Eidam) Winter. One culture developed from the C horizon and one from a surface soil plate incubated at 37°.

Aspergillus sydowi (Bain. & Sart.) Thom and Church. One isolation from a C horizon.

Cephalosporium spp.? Several isolations produced heads of spores as in the genus *Cephalosporium*, but could not definitely be referred to described species. In some cultures the mycelium became dark. It is quite possible that some of these isolations represent "oidial" stages on haploid mycelia of Hymenomycetes or other fungi.

Coremium sp.? One isolation produced coremia, but it was not identified

Dematium sp. A few cultures could be placed only tentatively in this genus.

Epicoccum nigrum Link. Two or three cultures were obtained; Dr. J. E. Machacek finds it also in cereal roots. The dark spores are 20–25μ in diameter, coarsely spiny, on conidiophores somewhat penicillately branched.

Fusarium ossicolum (B. & C.) Sacc. = ***F. equiseti*** (Cda.) Sacc. Form 1 Wollenw. Isolated twice.

Fusarium oxysporum Schlecht. var. ***aurantiacum*** (Lk.) Wollenw. Three isolations from Soil I.

Geomyces vulgaris Traaen. Several isolations, particularly from Soil I, fit this species (21). Cultures varied considerably in color, but all seemed to fit *G. vulgaris* better than Traaen's other species of *Geomyces* (*G. sulphureus*, *G. auratus* and *G. cretaceus*). Traaen's work has been more or less overlooked. It may be remarked in passing that *Sporotrichum carnis* is quite similar to *Geomyces vulgaris.*

Haplographium bicolor Grove. Five isolations from the surface horizon of Soils I and IV, four of which developed from isolations at 37° C. The fungus agrees with Bunting's illustrations in Mason (11).

Haplographium fuscipes (Preuss) Sacc. One isolation from a forest soil. It is well illustrated by Bainier (1). Von Hoehnel (9, No. 89) reports Bainier's fungus to be a form of *H. penicilloides* Fautrey, which is the same as *H. finitimum* (Preuss) Sacc.

Helminthosporium geniculatum Tracy and Earle. Three isolations from horizon A of Soil I. All *Helminthosporia* were determined by Dr. J. E. Machacek.

Helminthosporium ?rhabdiferum Berk. & Br. (possibly a *Dendryphium*). Two isolations from surface soils.

Helminthosporium tetramera McKinn. One isolation from surface of Soil IV.

Hormodendron viride (Fres.) Sacc. Occasional in the soils studied. Other *Hormodendra* are found also in soil: *H. cladosporoides* (Fres.) Sacc. is commonest; this we refer to its form *Cladosporium herbarum*.

Hyalopus ater Cda. group. One isolation from a surface soil.

Monilia implicata Gilm. & Abbott. A *Monilia*, apparently this species, was obtained from the surface soil of a wheat field.

Paecilomyces varioti Bain. (*Penicillium divaricatum* Thom). This fungus, found rather commonly in butter in Manitoba (4), appeared in two isolations, one from B and one from C horizons.

Penicillium cyclopium Westl. Rare in surface soils.

Penicillium ?dierckxii Biourge. One isolation from a forest soil.

Penicillium expansum (Link) Thom. This species, commonly reported from soils, was definitely identified only once from Manitoba soil, from the lower layer of a muskeg.

Penicillium flavi-dorsum Biourge. Also found in lower layer of peat.

Penicillium ?fuscum Sopp (as *Citromyces*). One culture from forest soil.

Penicillium guttulosum Gilm. & Abbott. This fungus was found to be common in the Forest Soils III and IV, particularly in sub-horizons A_2 to C.

Penicillium herquei Bain. & Sart. series. Found in meadow soil.

Penicillium nigricans Bain. group. A culture from forest soil.

Penicillium ?palitans Westl. From the lower layer of a muskeg.

Penicillium ?sublateritium Biourge. Also from the third layer of the peat.

Penicillium tardum Thom. From the B horizon of a forest soil.

Penicillium ?terlikowskii Zaleski. From the third layer of peat in a muskeg.

Penicillium thomi Zaleski (not *P. thomii* Maire). About 25 isolations from forest soil and peat. Close to *P. intricatum*, which is very common in Manitoba soils.

Periconia byssoides Pers. One isolation from meadow soil.

Periconia felina March. Thirty isolations from sub-horizons A_1, A_2 and B_1 of Meadow Soil I, Profiles 1 to 4.

Phoma hibernica Grimes, O'Conn. & Cumm. This fungus is common in butter in Manitoba (4). Three or four soil isolations produced pycnidia with pinkish spore masses and appeared identical with *P. hibernica* as it appears after some weeks in culture from butter. This fungus therefore may reach butter from the soil.

Sphaeronaema ?spinella Kalch. One isolation.

Sphaeropsis sp.? One isolation from the A_0 sub-horizon of a forest soil produced pycnidia with dark spores 14–17 × 6–8μ.

Sporotrichum pruinosum Gilm. & Abbott. Two isolations.

Stachybotrys sp. One isolation from the B horizon produced rough conidiophores and large rough dark spores 15–21 × 8–13μ. No description was found to fit this species.

Stemphylium macrosporoideum (Berk.) Sacc. One isolation, determined by Dr. Wiltshire. Eleven other isolations of *Stemphylium* could not be determined.

Tubercularia ?vulgaris Tode. One isolation produced pink sporodochia and hyaline spores resembling those of *T. vulgaris*.

Verticillium ?nubilum Pethybr. A half-dozen isolations from surface soils produced verticillate conidiophores with spores 4–8 × 2–3μ. The cultures become dark with chlamydospores measuring 6–10 × 6–8μ. These cultures agree fairly well with the description by Pethybridge (13).

Volutella roseola Cke. One isolation from a surface soil agrees in detail with this fungus reported from soil by Bayliss Elliott (2).

Analysis of the Micro-organisms in a Profile

It seems unnecessary to give the complete protocols of fungi from the 12 profiles studied, since they are analyzed in subsequent sections. We give as an example, however, the identifications from Profile 8 of Soil III.

I. Fungi isolated by the standard technique, i.e., aerobically at 25° C., from which all colonies were picked from representative plates for identification (see 3, pp. 253–254).

Sub-horizon A_0: *Absidia orchidis*, 2 cultures; *Acrostalagmus albus* var. *varius*, 2; *Alternaria* sp., 2; *Aspergillus okazakii*, 1; *Coniothyrium* sp., 1; *Geomyces vulgaris*, 2; *Mortierella isabellina* var. *ramifica*, 4; *M. vinacea*, 5; *Mucor ?sylvaticus*, 2; *Penicillium braziliense*, 3; *P. ?canescens*, 1; *P. chrysogenum*, 2; *P. janthinellum*, 15; *P. lilacinum*, 2; *P. thomii*, 10; *P.* spp., 6; *Trichoderma album*, 3; *T. lignorum*, 4; *Verticillium ?glaucum*, 1; *V. terrestre*, 1.

Sub-horizon A_1: *Cephalosporium* sp., 1; *Penicillium rugulosum*, 1; no spores produced, 1.

Sub-horizon A_2: *Absidia spinosa*, 2; *Cephalosporium acremonium*, 2; *Cladosporium herbarum*, 1; *Cylindrocarpon heteronemum*, 5; *Mortierella vinacea*, 3; *Mucor racemosus*, 1; *Penicillium guttulosum*, 17; *P. janthinellum*, 2.; *P. restrictum*, 4; *P. restrictum* variety, 5; *P. thomii*, 1; *P.* spp., 7; *Phoma* sp., 1; *Trichoderma album*, 1; *T. koningi*, 4; *Verticillium* sp., 4; Unknown, 5.

Horizon B: *Cephalosporium* sp., 9; *Cylindrocarpon candidum* var. *majus*, 1; *C. heteronemum*, 1; *C. macrosporum*, 1; *Gliocladium roseum*, 2; *Mortierella vinacea*, 2; *Penicillium guttulosum*, 13; *P. intricatum*, 1; *P. restrictum*, 2; *P. restrictum* variety, 21; *P. thomii*, 3; *Trichoderma album*, 2; *T. glaucum*, 1; *T. koningi*, 1; Unknown, 3.

Horizon C: *Cladosporium herbarum*, 2; *Cylindrocarpon macrosporum*, 2; *Geotrichum candidum*, 2; *Mortierella elasson*, 5; *M. vinacea*, 1; *Penicillium guttulosum*, 12; *P. restrictum*, 1; *P. thomii*, 1; *Trichoderma album*, 1; *T. lignorum*, 4; Unknown 2.

II. Fungi isolated at 37° C., i.e., the dilution plates were incubated at 37° and representative plates picked as before.

Sub-horizon A_0: *Aspergillus fumigatus*, 3; *A. nidulans*, 1; *A. niger*, 1; *Trichoderma koningi*, 5.

Sub-horizon A_1: *Trichoderma koningi*, 11.

Sub-horizon A_2: *Trichoderma koningi*, 2. Horizons B and C: no fungi obtained.

III. Fungi isolated anaerobically, i.e., the dilution plates were incubated in an atmosphere of CO_2.

Sub-horizon A_0: *Mucor* spp., 3; *Trichoderma album*, 3; *T. lignorum*, 5; no spores, 1.

Sub-horizon A_1: *Penicillium* sp., 3;

Sub-horizon A_2: *Mucor hiemalis*, 2; *Penicillium* sp., 4; *Trichoderma album*, 2; *T. lignorum*, 1; *Zygorhynchus moelleri*, 1.

Horizon B: *Cylindrocarpon candidum* var. *majus*, 4; *Trichoderma lignorum*, 1; *Verticillium ?glaucum*, 1.

Horizon C: *Colletotrichum* sp., 3; *Trichoderma koningi*, 1.

IV. Fungi isolated at about 6° C., i.e., the dilution plates were incubated in a refrigerator.

Sub-horizon A_0: *Mucor ?sylvaticus*, 1.; *Penicillium janthinellum*, 7. A_1: no data.

Sub-horizon A_2: *Cladosporium herbarum*, 1; *Cylindrocarpon macrosporum*, 1; *Mortierella ?vinacea*, 2; *Mucor hiemalis*, 2; *M. ?sylvaticus*, 1; *M.* sp., 3; *Periconia felina*, 1; *Trichoderma album*, 1; *? Verticillium* sp., 3.

Horizon B: *Cylindrocarpon macrosporum*, 3; *Mortierella vinacea*, 5; *Penicillium lilacinum*, 1; Horizon C: *Cylindrocarpon macrosporum* 3; *Mortierella elasson*, *1;* no spores, 1.

It may be mentioned that the data as to the numbers of fungi per gram of soil were obtained from the original isolation plates. The relative frequencies of different species of fungi were obtained by transferring all young colonies from representative plates to test tubes. These were incubated and subsequently the fungi were identified insofar as was possible.

The Important Soil Fungi of Manitoba

In an earlier paper (3) 121 different fungi were reported from Manitoba soil. In this paper 56 are added. Some of these 177 fungi are determined to genus only. The preceding paper (3) presented an analysis of the relative frequency of the species identified from 2565 colonies transferred "at random" from the dilution plates. In the work on soil profiles here reported 2163 colonies were studied similarly, so that information is available now as to the distribution among species of a "random sample" of 4728 Manitoba soil fungi. An analysis of the more important genera and species follows (see also Table I).

The "standard" isolation technique (23, see also 3) has the advantages of providing media upon which many kinds of fungi will grow, of making available comparable data on numbers of fungi per gram of soil and on the species present in various profiles or in various regions of the world. There are certain obvious disadvantages, however, to the routine methods of isolating fungi from the soil. These may be eliminated in part by plating from higher dilutions of soil to obtain species which grow more slowly, by incubating at high and low temperatures and under anaerobic conditions, and by the use of special media for certain fungi. All these methods have been utilized in Manitoba, although efforts have not yet been made to obtain species of *Pythium*, Saprolegniales, or Hymenomycetes from the soil.

PHYCOMYCETES

Mortierella has proved to be the most abundant Phycomycete in the soil profiles examined. *Mortierella isabellina* var. *ramifica* was previously (3) found twice in surface layers of forest soil: now it has been identified 40 times, from forest and especially from peat, in both of which it occurs usually in the upper horizons. It was not found in Soil I. *M. elasson* was especially common in the C horizon of Meadow Soil I, and to a lesser extent in the B_2; it was found also 7 times in the C horizon of forest Soil III, but not in the A horizon of either soil, and not in peat. *M. vinacea* was common in forest soils and fairly common in peat. It was usually found in the upper sub-horizons. Thus the evidence suggests that species of *Mortierella* may be characteristic of certain types and horizons of soil.

Species of *Mucor* are fairly common in the A horizon of forest soil, rare in the B horizon, and were not isolated from any C horizon. They did not develop from Soil I. Specific determinations in the genus *Mucor* are difficult.

Absidia was found somewhat more commonly than *Mucor* in the profiles examined. *A. spinosa* was isolated 29 times, 22 of which were from Soil I. *A. orchidis* was obtained 15 times, 12 of which were from forest soil. *Absidia* like *Mucor*, is more common in the A horizon.

Rhizopus was isolated infrequently from the soil profiles studied, but was found rather commonly in surface soil in our previous work (3). *Zygorhynchus* is rare: it has been obtained only seven times from Manitoba soil. The few other Mucorales found may have been only "accidentally" present in the soil.

Plant pathologists in western Canada have demonstrated that species of *Pythium* occur in soil and cause injuries to crops. *Pythium* does not appear, however, in ordinary soil isolations. A special technique would be required to demonstrate the frequency of this important genus in the soil.

ASCOMYCETES

Isolations producing asci readily in culture were obtained from soil rarely, and those found usually were not true soil fungi. However, certain Sordariaceae such as *Chaetomium* spp. grow on debris and play a part in its disintegration in the soil. The larger Ascomycetes, such as Helvellales and Pezizales, often grow on decaying leaves or rich soil, but are rarely obtained in soil cultures. The points mentioned in the next paragraph apply also to many of these Discomycetes and to certain Pyrenomycetes.

BASIDIOMYCETES

The isolation technique ordinarily used by the soil microbiologist yields little information on the flora of Hymenomycetes and Gasteromycetes known to be abundant in the soil, particularly in forests. Mycelium showing clamp connections was conspicuous by its absence in the cultures studied. Some of the sterile mycelia or certain cultures resembling *Cephalosporium* may have represented haploid stages of Basidiomycetes. A ramble through the woods or even over the prairie during a damp autumn in Manitoba gives one the impression that the large Basidiomycetes constitute the predominant fungus flora, and much mycelium can be traced from the bases of mushrooms, etc. into the leaf mold, the soil, or to twigs or roots. We have records of about 570 species of Agaricaceae in Manitoba, the large majority of which grow "on the ground". About 100 other Clavariaceae, Hydnaceae, Boletaceae, Lycoperdales, etc. also are to be found growing from the soil or fallen leaves; and about 200 other Polyporaceae, Thelephoraceae, etc., are to be found on wood. This large assemblage of fungi, giants as compared with the tiny (but abundantly sporulating) colonies of *Penicillium* or *Trichoderma* in the soil, should be taken into consideration in appraising the soil fungi. These Basidiomycetes play a very important part in the early stages, at least, of the disintegration of woody tissue and leaf mats, but the fungi isolated by plating soil are more truly "soil fungi" in that they commonly live upon organic matter sufficiently decomposed to be considered part of the soil itself.

Spores of rusts and smuts fall in numbers upon the soil, but only rarely does one obtain a culture which can be identified as one of the Ustilaginales.

FUNGI IMPERFECTI

The great majority of fungi isolated from the soil must be placed in the Fungi Imperfecti, as one very rarely finds an ascus stage developed in the cultures kept in the laboratory.

Penicillium constituted nearly half the isolations from various horizons (920 cultures in a total of 2163), and was found in slightly over half the isolations previously (3) recorded (1404 cultures in a total of 2565). *Penicillium* is a very difficult genus. Accordingly we have not recorded species unless first determined by Thom. As the work progressed greater proficiency in identifying the commoner soil types by comparison with the determined cultures was attained, with the result that each lot sent to Dr. Thom tended to increase in difficulty and a score or so of forms were encountered which did not fit previously known species. It seems evident that some species of *Penicillium* are characteristic of certain types or horizons of soil: thus five species listed above were found only in the third layer of a muskeg; *P. guttulosum* was found to increase with depth in forest soil; *P. thomii* occurs throughout forest soil, rarely elsewhere; *P. terrestre*, so common in soils that had grown wheat (3, p. 264) was found only five times in the virgin soils herein analyzed; P. *chrysogenum* also appears to be commoner in cultivated soils; *P. intricatum* was found only occasionally in forest, but abundantly in most other soils; *P. lilacinum* was found especially commonly in Meadow-prairie Soil I; *P. rugulosum* was found fairly frequently in peat, where other species common in forest and field were mostly absent.

Aspergillus was not found to be common in the horizons studied, and when found was usually in the A horizon (49 isolations from A, and five in B and C together). Further evidence that *Aspergillus* prefers high temperatures is provided by the results of isolations made at 37° C. from which 27 of the 49 isolations developed. No *Aspergillus* developed in plates incubated in the refrigerator or anaerobically. *A. niger*, so common in southern soils, appeared only once in the course of the present study, from the A_0 of a forest soil incubated at 37° C. This corroborates our previous finding (3, p. 259) that *A. niger* is very rare in Manitoba. *A. fumigatus* was obtained ten times from dilutions incubated at 37° C. *A. flavipes* again was the commonest *Aspergillus*, appearing 25 times, 23 of which were from Soil I and 16 of these from the 37° C. isolations. *A. flavus* was found once in each horizon of Soil I. *Aspergillus* has been isolated only 104 times from Manitoba soil, whereas *Penicillium* has appeared 2324 times.

Trichoderma is much commoner in Manitoba soil than is *Aspergillus*, and is of particular interest because it is known to be "one of the most active groups of soil fungi decomposing celluloses, proteins, pectins and other complex organic substances" (23, p. 703) and *Trichoderma* has recently attracted considerable attention as a parasite of other fungi as was first recorded by Weindling (25) and independently found by the writers (3). Weindling (26–29), and others (8) have studied this parasitism further and have sought to prevent certain plant diseases by adding *Trichoderma* to the soil. The recorded species of *Trichoderma* do not appear to be clear-cut (see 10), but we have identified species as follows from the profiles studied: *T. album*, 19 isolations; *T. glaucum*, 15; *T. koningi*, 91; *T. lignorum*, 95. *Trichoderma* is the commonest fungus on plates incubated anaerobically, and is rather commonly found on those incubated at 37° C., but only once did we get a *Trichoderma* (*T. album*) on plates held in the refrigerator. *Trichoderma* is especially common in forest soils throughout all horizons and is fairly common in peat. It was surprising, however, to find it only eight times from the four profiles of Soil I and not at all from the alkalinized Soil II. Weindling and Fawcett (29) found *Trichoderma* more active in acid soils.

Cylindrocarpon has proved to be a rather common fungus in most profiles studied, having appeared 99 times: in A horizon, 37, in B, 46, and in C, 16 isolations. Its greater prevalence in the present isolations than in those reported previously for Manitoba ((3) 24 isolations only) is due perhaps to its greater abundance in sub-surface horizons. *Cylindrocarpon* grows readily at low temperatures, being the commonest fungus on plates incubated in the refrigerator. It is rather common on plates incubated anaerobically, but was not obtained at 37° C. *C. macrosporum* was obtained 51 times; *C. candidum* 11, and its var. *majus* 21 times; *C. heteronemum* 14 and *C. didymum* 2. *Cylindrocarpon* was not obtained from or below peat.

Fusarium was found sparingly in the profiles of virgin soil studied (34 cultures, whereas the surface soils previously reported (3), most of which were under cultivation, yielded 128). *Fusaria* were commonest in Meadow-prairie Soil I (28 cultures); rare in forest soils (5 cultures), and only one culture was obtained from peat. Most of the cultures were from the A horizon, although they were obtained from B and C also. *F. oxysporum* and its varieties were the commonest forms isolated, but *F. coeruleum*, *F. poae*, *F. Solani* var. *martii*, *F. scirpi* var. *acuminatum*, *F. equiseti* form 1, and *F. sporotrichoides* were also found in the profiles. Several cultures of *Fusarium* were obtained from plates incubated anaerobically, but none at 37° C. Reinking and Manns (15, 16, 17) have recorded the *Fusaria* from several tropical soils, and have classified the various species isolated as "soil fungi" or "soil invaders." The lists of soil *Fusaria* from the tropics resemble the list from Manitoba soil; as Reinking (14) states, "many of the common *Fusaria* present on plants and in the soil of the temperate zone are distributed in the tropical regions." The species mentioned above from virgin Manitoba soil may be considered "soil *Fusaria*"; some of those previously (3) isolated from cultivated soils, such as *F. culmorum*, are apparently "soil invaders." Ziling (30) found *Fusaria* in Siberian soils very similar in numbers and kinds to those here reported.

Helminthosporium sativum causes serious root rots of cereals; it was isolated four times from the A horizon of Meadow-prairie Soil I, but from no other virgin soils. It is therefore a native of Manitoba grass land. Forty samples previously analyzed (3) of soil cropped for years with wheat showed about the same sparse occurrence of *H. sativum*. It is probably prevented from accumulation in many cultivated soils by the activities of other soil microorganisms, such as *Trichoderma*. *Helminthosporium* seems to be of little importance in the soil, except for the potential parasitism of *H. sativum* on the Gramineae; but it may be that some or all species do not sporulate readily in the soil.

Rhizoctonia solani is known to vegetate in the soil as a sterile mycelium. It is therefore not likely to appear often on dilution plates from soil. Cultures with the mycelial and sclerotial characters of *R. solani* were obtained six times from virgin soil. This fungus, some strains of which are actively parasitic to higher plants, must therefore be fairly prevalent in such soils. It often causes injury to crop plants in Manitoba.

Cephalosporium has occurred as about 2% of all Manitoba soil fungi; 57 isolations attributed to this species were found in the profiles studied, 17 of which developed under anaerobic conditions but none at high or low temperatures (see Table I). This fungus appears rather evenly distributed through all soils and all horizons. *Cephalosporium* is an uncertain genus in that conidial or "oidial" stages of several fungi, including certain Hymenomycetes, may resemble the spore heads of *Cephalosporium*. Bayliss Elliott (2), for example, reports a conidial stage of *Volutella roseola* to agree with *C. acremonium*. *C. curtipes* was identified 11 times from the soil profiles, *C. humicola* 9, *C. acremonium* 5, undetermined species 32.

Verticillium occurs occasionally in soil (24 cultures, or 1% in the profiles). It is found especially in the A horizon of forest soil. The soil *Verticillia* need critical taxonomic study.

Alternaria is found occasionally in virgin soils (19 cultures from profiles) and this genus was noteworthy for its even, though sparse, distribution through all soils studied and in all horizons; it developed five times in anaerobic cultures and once each at 37° and at 6° C. Three cultures were identified as belonging to the *A. tenuis* group, the others remain unidentified. The related genus *Stemphylium* is somewhat less common (12 cultures) but was found occasionally in each type of soil.

Cladosporium herbarum was obtained only 16 times from the virgin soil profiles, whereas 69 cultures were obtained previously (3) from surface soils most of which were cultivated. Four cultures of the 16 developed at 6° and none at 37° C. or anaerobically. *Cladosporium* was found in both meadow and forest soils, from A to C horizons.

Phoma and *Coniothyrium* (32 and 22 isolations respectively) are rather common throughout both meadow and forest soils. These are interpreted to arise from spores from pycnidia of various fungi growing on decaying roots and other plant parts.

Monotospora daleae Mason (11) deserves mention because it was isolated 15 times from the A and B horizons of Meadow Soil I and not from any other soil. It was previously (3, where it is recorded as *Mycogone nigra*) found 18 times, principally from a wheat field. These figures suggest that *M. daleae* develops in soil containing parts of Gramineae.

Metarrhizium sp., previously (3) reported as common in soil of wheat fields, was conspicuous by its absence, save for one doubtful isolation, from the virgin soils studied. Dr. J. E. Machacek finds the same *Metarrhizium* occasionally in diseased cereal roots.

Periconia felina is noteworthy because it appeared 31 times from the A and B horizons of Meadow Soil I, but has not been obtained elsewhere in Manitoba.

Gliocladium occurs throughout meadow and forest soil and can be considered a true "soil fungus." *Geomyces vulgaris* perhaps is also a widespread inhabitant of soils. *Acrostalagmus* spp. and *Hymenula affinis* are occasionally obtained from various soils in Manitoba.

Other Fungi Imperfecti isolated from the soil, such as species of *Botrytis*, *Geotrichum*, *Monilia*, *Scopulariopsis*, *Sporotrichum*, *Haplographium* and *Colletotrichum*, have been obtained so infrequently in Manitoba that little can be said about their possible roles in the soil. A number of other fungi, such as species of *Cytospora*, *Dactylium*, *Tubercularia*, and *Hyalopus*, evidently appear in cultures from the relatively few spores that have fallen upon the soil from a saprophytic or parasitic growth upon the plants above, or even within, the soil. When it is remembered that an average surface soil in Manitoba contains about 125,000 viable spores (including occasionally bits of mycelium) *per gram*, it becomes clear that the extraneous spores which fall upon the soil must make up but a minute fraction of the population of fungi present.

Undetermined fungi inevitably remain as a residue from large numbers of isolations from soil. Nearly half of the 170 transfers which were not determined at the time of writing the previous paper (3, p. 267) have now been determined, but, for lack of spores or other reasons, no identification was made of 168 of the 2163 fungi isolated from soil profiles.

Table I summarizes all species which were isolated 12 or more times (*i.e.*, 0.5% or more) from the soil profiles studied. When the genus name alone is given, the entries refer to all cases in which the total isolations were 12 or more, but not that number under any one species of the genus. Table I also includes the number of isolations, of the species of genera included, obtained from transfers from plates incubated at 37° C., at 6° C., or anaerobically.

Fungi in Different Soil Horizons

The surface sub-horizon, A_0 or A_1 as the case may be, receives many spores from the air, so that fungi extraneous to the soil are most likely to be obtained there, but in small numbers. In Table I most or all such fungi have been eliminated. *Aspergilli* and certain *Penicillia* and other fungi show a tendency to decrease with depth. On the other hand *Mortierella elasson*, *Cylindrocarpon*

TABLE I

NUMBER OF ISOLATIONS OF COMMONER FUNGI FROM SOIL HORIZONS

Fungus	Soil I					Soil III				
	A_1	A_2	B_1	B_2	C	A_0	A_1	A_2	B	C
Absidia orchidis				2	1	4				
Absidia spinosa	12	5	2	3		5		2		
Mortierella elasson		1†	2	19	30 7† 2§					6 1†
M. isabellina ramifica						6	6			
M. vinacea	1*					7	6	3	3	1
Mucor spp.	2 2§	2	1* 1†			3		1§	1§	2
Alternaria spp.	2 2§	2	1* 1†			3		1§	1§	2
Aspergillus flavipes	3 7*	4 9*								
A. spp. all others	2 5*	1	1	1	2	4 5*	2			1
Cephalosporium spp.	1	2	1 7§	1			2	9	13	1
Cladosporium herbarum	4 3†						1	5 1†		2
Coniothyrium spp.		2 2§	4			3				1
Cylindrocarpon candidum var. *majus*	1	1 1§	5 1§	4	3		•		1 4§	
C. macrosporum	2 4†	1	6 4†	10 2§	3		1 . 1†	1 1§ 3†	4	4 3†
C., other species	4	5	5 1§ 2	2			5	1		
Fusarium spp.	18 7§ 1.	2				1§			1§	
Gliocladium spp.		8	7			1			2	
Monotospora daleae	1 1†	6	6	1						
Pencillium guttulosum					1		1	17	13	12
P. intricatum	28 2†	61 6†	2 2†	2 1†				1	8	
P. janthinellum	38	52 7†	21			23		2	1	
P. lilacinum	17 1§	6	5	2		2			1†	
P. purpurogenum	1					7		1*	6 5*	
P restrictum var.								6	21	1
P. rugulosum						1				1
P. thomi							1	1	3	
P. thomii				1		19	2	1	15	18
P. spp., all others	4 1§	7 1†	8	6 2†	4	17 1*	8	8	11	6

**Developed at 37° C.* *†Developed at 6° C.* *§Developed anaerobically.*

TABLE I—*Continued*

NUMBER OF ISOLATIONS OF COMMONER FUNGI FROM SOIL HORIZONS—*Continued*

Fungus	Soil I					Soil III				
	A_1	A_2	B_1	B_2	C	A_0	A_1	A_2	B	C
Periconia felina	2 1†	18 2§	7							
Phoma spp.	12	5	1	1		7	1	1		
Trichoderma album			1†			4 3§		3 2§	3 1§	1
T. glaucum								2	1	
T. koningi	1§					5 7* 3§	10 1* 1§	6 2* 7§	3 2§	3 3§
T. lignorum	1§	2	3			8 1* 12§	5		12 2§	9
Verticillium spp.			1			5		5	1§	1§

Fungus	Soil IV					Soil V		
	A_0	A_1	A_2	B	C	1	2	3
Absidia orchidis	5 1§			2				
Absidia spinosa								
Mortierella elasson	2?				1§			
M. isabellina ramifica	3			1		10	11	3
M. vinacea	8	10	4	2		7	3	3
Mucor spp.		1	1 1*				1	
Alternaria spp.		1	1 1*				1	
Aspergillus flavipes		2						
A. spp. all others		2	2			1		
Cephalosporium spp.	6	1	6§	2	4§		1	
Cladosporium herbarum								
Coniothyrium spp.	9 1*							
Cylindrocarpon candidum var. *majus*								

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*Developed at 37° C. †Developed at 6° C. §Developed anaerobically.

TABLE I—*Concluded*

NUMBER OF ISOLATIONS OF COMMONER FUNGI FROM SOIL HORIZONS—*Concluded*

Fungus	Soil IV					Soil V		
	A_0	A_1	A_2	B	C	1	2	3
C. macrosporum								
C., other species					1 1§			
Fusarium spp.				1 1§	1§			1
Gliocladium spp.	2§		1§	1§				
Monotospora daleae								
Penicillium gutlulosum	2	10	21	23 2§	23 14§			
P. intricatum	5							
P. janthinellum	21	4	1		5			
P. lilacinum								
P. purpurogenum								
P. restrictum. var.		1		5				
P. rugulosum						9	7	7
P. thomi				12	5			14?
P. thomii	19	7		2		19*		1
P. spp., all others	9 1§	7 3§	5 5§	4 1§	1 1§	34 5§	14 4§	44 4§
Periconia felina								
Phoma spp.	3 1§							
Trichoderma album				1§				
T. glaucum	1§	1 1§		2§		2§	2 3§	
T. koningi	6 1* 2§	2 1§	1 5* 2§	1 2§	2§	3 3* 1§	2	1
T. lignorum	1 8§	4 7§	7 3§	2 2§	1§		4 1§	
Verticillium spp.		8	2	2				

**Developed at 37° C.* .*Developed at 6° C.* §*Developed anaerobically.*

macrosporum, and *Penicillium guttulosum* showed distinct increases in deeper soil horizons. As might be expected, however, there is usually no striking difference in the fungus-flora of different horizons.

Horizon C begins at 17 to 27 inches in Profiles 1 to 10. In Profiles 11 and 12, the peat bog, the third layer (not a horizon) began at 58 and 70 inches. *Aspergilli* were not common in C or B horizons, and the *Penicillia* were rare in the C horizon of Meadow Soil I. The only *Penicillium* common in C horizons of Forest Soils III and IV was *P. guttulosum*. Several species of *Penicillium*, however, made up the majority of the organisms in the lower layer of peat. Anaerobic and low temperature fungi are more common in the C and B horizons than in the A horizon. It is possible that strains of fungi differing physiologically and even morphologically may develop during a long sojourn deep in the soil. We were at first inclined to think that fungi from lower horizons more frequently failed to produce spores than those from upper horizons, but such is not really the case, although spore production may be delayed. Only 16 isolations from C horizons could not be identified. Some of these produced spores.

The Fungi in Peat

One striking feature of the peat studied was the marked decrease of fungi with depth. Waksman and Purvis (23) found the same phenomenon, no fungi being obtained in peat from Maine at 30 inches and below. *Mortierellae* were common in peat in Manitoba (see Table I). *Penicillia* constituted 60% of all fungi isolated, but were for the most part species not found in other soils studied. Peat is, of course, very different from ordinary soil and has a different flora of fungi.

Anaerobic Fungi in the Soil

Lower horizons of heavy soils must often be deficient in free oxygen. Yeasts and certain other fungi are known to be capable of living anaerobically, or at least in media with low oxygen content. We have not seen, however, reports on the anaerobic fungi of the soil.

TABLE II

Numbers and percentage of facultatively anaerobic fungi per gm, as compared with total fungi

Profile	Horizon	Total fungi per gm.	Anaerobic fungi	
			Number per gm.	Percentage
6	A_0	117,000	1120	0.96
	A_1	15,625	1010	6.5
	A_2	4,250	590	13.9
	B	995	540	54.3
	C	120	50	41.7
9	A_0	205,000	1326	0.65
	A_1	65,000	970	1.5
	A_2	7,500	1650	22.0
	B	14,750	2380	16.2
	C	1,840	680	37.0

Anaerobic conditions were obtained by introducing carbon dioxide into a chamber until the air was displaced. Soil dilution plates were incubated at the bottom of the chamber, closed except for two tubes, through one of which carbon dioxide constantly entered slowly, the other serving as exit tube. The figures given by Timonin in the previous paper (20) show that from 20 to 2850 anaerobic fungi were found per gram in Soils I, III and IV, and up to 35,000 per gram in peat. In the peat about 10 to 20% of the total fungi in all three layers are facultatively anaerobic, but in the other soils there is a noteworthy percentage increase of anaerobes with depth. This is brought out in Table II.

As can be seen in Table I, relatively few *Penicillia* capable of developing anaerobically were found. Thom (19, p. 84) states that "Absence of free oxygen practically stops the growth of *Penicillium*." A few soil forms, however, can make good growth in an atmosphere of carbon dioxide. No *Aspergilli* were obtained anaerobically, but *Trichoderma* appeared commonly. Species of *Cylindrocarpon*, *Fusarium*, and Mucorales were the other fungi principally found. *Verticillium*, *Alternaria*, and *?Colletotrichum* were also obtained.

All fungi which developed in the atmosphere of carbon dioxide and which were transferred to test tubes for study, were found to grow readily aerobically. The faculty of growing anaerobically must aid soil fungi materially in deeper soils and in peat bogs.

Soil Fungi Which Develop at 37° C.

Soil dilutions from several horizons were incubated at 37° C. These plates showed a striking prevalence of *Aspergilli*. *Trichoderma koningi* also can flourish at a high soil temperature. Relatively few *Penicillia* developed at 37° C. (see Table 1). Certain fungi not usually found in soil cultures appeared, such as *Humarina*, *Xylaria*, and *Haplographium*. Most of the high temperature fungi were found at or near the surface of the soil which of course is subjected to the highest summer temperatures.

Soil Fungi Which Develop at Low Temperatures

Dilution plates incubated in a refrigerator at about 6° C. developed *Cylindrocarpon*, Mucorales, certain *Penicillia*, less frequently *Cladosporium*, and rarely other fungi. No *Aspergilli* and only one *Trichoderma* was obtained (see Table I). The low temperature fungi show an increase, relative to total fungi, with greater depth of soil. It is to be noted from the tables in the preceding paper (20) that low temperatures commonly prevail in the lower horizons of soil in Manitoba: for example, 9° C. in the C horizon below peat on July 19 and 11° C. in B and C horizon of Profiles 9 and 10 on the same date. On May 15 the C horizon was still 0° C. in Profile 2.

Conclusion

Some years ago the question arose as to the occurrence of a definite fungus-flora of the soil. The answer seems clearly in the affirmative. Indeed, Waksman (22) by 1916 was able to postulate a "typical soil flora" of fungi which has in general been verified by subsequent work in various parts of the world. Jensen (10) records a list of fungi from soils in Denmark which is very similar to the list from Manitoba; both show the relative infrequency of *Aspergilli* in northern areas. Ziling (30) finds a flora of soil fungi in Siberia very like that in Manitoba and elsewhere. Miss Nobles (12) isolated 24 genera of fungi from the soil of southern Ontario, all but one or two of which have been found in Manitoba, and in about the same relative abundance. Many soil fungi are very widely distributed, as mentioned above for *Fusaria*. Chaudhuri and Sachar (5) report about 30 species of fungi from Punjab, India: about half of these have been found in Manitoba. In India *Aspergilli* are no doubt common soil fungi: one-third of the species recorded by Chaudhuri and Sachar belong to this genus. Weindling (27) makes the interesting suggestion that one reason for the prevalence of certain fungi in the soil is their ability to overcome their competitors and even to use their hyphae as food.

Despite the facts that many soil fungi are very widely distributed and the flora of any soil constitutes a very complex and fluctuating population, there is evidence that certain species of fungi may be characteristic of certain types or horizons of soil. This paper attempts to evaulate the significance of various species by considering their relative abundance in different horizons under high, intermediate or low temperatures of culturing, presence or absence of oxygen, and such other data as are available.

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A COMPARISON OF THE BARGE WITH OTHER METHODS OF HARVESTING WHEAT[1]

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Abstract

In 1932, in the Edmonton district, under almost ideal harvest conditions, samples harvested by the barge method were equal to or better in grade and quality than those harvested by the binder, swather or straight-combine methods. In 1933, under more adverse conditions, the barge samples were the poorest in grade and baking quality. There was some loss of material due to molding and sprouting in the barge stacks. Bin storage markedly improved the grade and quality of barge samples.

Wheat combined at 14.0% moisture content did not heat or increase in moisture content significantly during storage.

Introduction

In 1932 and 1933 a comparison of the barge method of harvesting (5) with the binder, swather and straight-combine methods was undertaken as part of an investigation on the effect of the method of harvesting on the moisture content and grade of wheat, and was extended to include the determination of milling and baking quality.

The more extensive studies on the comparison of stook-threshed, swath-combined and straight-combined samples have been reported by Larmour, Geddes and Cameron (3) and Larmour and Geddes (4). The various samples used in these studies were collected in field surveys, and hence were not of comparable origin. The results showed the highest percentage of tough and damp samples harvested by the straight-combine method and the lowest by the binder and thresher method. In 1932, the highest average grade was obtained with the binder and thresher samples and the lowest with the swath-combine samples. In 1933, these results were reversed, but the differences in the average grades were smaller than in 1932. As a consequence, the two year average gave the same relation as the 1932 results.

During the part of the harvest season over which the experiments now to be reported were carried out at Edmonton in 1932 there was but little rain, the temperature in general was high and the humidity low. Drying conditions were therefore close to ideal. During the same part of the season in 1933, there was considerable rain and there were periods of relatively low temperatures and high humidities. A comparison of the results obtained with the various methods of harvesting for the two years gives some idea of what might be expected in areas usually characterized by one or the other type of weather conditions.

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Equipment and Material

The equipment used in harvesting the binder and barge samples was the same for the two years. The former were cut with a seven foot binder, which was also used with a 7 × 6 × 6 ft. barge in harvesting the barge samples. This type of barge equipment is somewhat unsatisfactory because more straw has to be included in the material stacked than where a header, or a binder converted to function as a header, is used, and the barge is too small to permit the most efficient stacking. Better equipment was not available, so more than usual caution was exercised in building the stacks.

The material used in both years was Marquis wheat grown on a farm located about seven miles east of Edmonton. The land was slightly rolling, but the portions of fields used were fairly uniform. In both years part of the wheat was grown as the second, and part as the first, crop after fallow. In both cases, that part of the crop on stubble land is designated Field 1 and that on fallow land Field 2.

In 1932 the fields were harvested in strips of equal width by each method. The grain in Field 1 did not ripen very uniformly, there being a small percentage of relatively green heads among the more generally ripe crop. The moisture range of grain from individual heads at the time of cutting was from 17.8 to 41.2%, the average being 25.9%. The straw was in general short and dry. The grain was combined when the moisture content of the kernels was 14.2%.

The crop on Field 2 was much heavier than that on Field 1 and development was more uniform. The moisture range of grain from individual heads was from 21.7 to 30.7%, the average being 23.6%. Harvesting was 12 days later than in Field 1. The grain was combined when the moisture content of the kernels was 13.9%.

In 1933 the first three acres was cut around Field 1 with the binder, the second three acres with the barge, the third with the swather and the remainder of the field with the combine. Harvesting by the first three methods was spread over three days. The moisture range at the time of cutting was from 15 to 53%, but the extremes represented only small spots in the field. The average moisture content was 31%. The straw was short and dry and there were but few green spots in the field. The yield of grain was about 30 bushels per acre. When the moisture content of the standing grain reached 15% combining was started, but owing to an accident with the machinery it was not completed until the next day, when the moisture content was 13.7%.

Field 2 was smaller than Field 1 and only the first three methods of harvesting were used. The grain was cut in a manner similar to that used for Field 1 except that the areas harvested with the binder and the swather were reduced to one-half acre each. The remainder was harvested with the barge. The binder and swather samples were cut when the average moisture content of the grain was 42%, but barging was not completed until the next day when the average moisture was 39%. Any advantage in maturity is therefore in favor of the barged sample. The moisture range in this field was from 24 to 51%, the straw was much heavier than, and not as dry as, that of Field 1. The yield of grain was about 35 bu. per acre.

In 1932 only the combined grain was studied after threshing. Wheat to a depth of approximately five feet was placed in a bin and temperature and moisture determinations made for a few days.

In 1933, the barge, swather and straight-combine samples from Field 1 and the barge sample from Field 2 were stored in four sections of a large bin. Individual bins, approximately 8 × 4 ft. in size, and separated from those adjoining by two-inch air spaces, were filled with grain to a depth of 4.5 ft. These lots of wheat were stored from the time of harvesting until March 1934, when they were re-sampled. The samples collected at harvest were laboratory stored, and both these, and those collected after bin storage, were used in testing for quality.

Methods

Moisture

All moisture determinations were made on approximately 80 heads taken at different places in the field of standing grain, and from different stooks, swaths or stacks of cut grain. The heads were placed in air-tight cans, taken to the laboratory, threshed out immediately, dried at 100° C. for five hours, ground and dried again at 130° C. for one hour. In sampling the barge stacks, heads were taken from three places in the stacks, a foot from the top, at the centre, and a foot from the bottom. Only heads from the inner part of the stacks were taken. Binned grain was sampled at several different places.

Temperature and Humidity

In 1932, temperature and humidity readings were taken at as near 3 p.m. as possible, and the values recorded are for this time. Maximum thermometers were left in the stacks for 1½ hr. and then read. In 1933 the temperatures recorded are the maximum for the day. The relative humidity was determined with sling psychrometers as near 10 a.m. as possible. Thermometers were placed in the barge stacks for 24 hr. before being read. The values therefore represent the maximum temperatures during the preceding 24 hr. Temperatures of binned wheat were determined by placing stick thermometers in the middle of the bins, six inches from the top, at the centre, and six inches from the bottom of the grain. The thermometers were removed only to take the readings and to lower the mercury columns.

Grades

All grading was done at the Edmonton office of the Western Grain Inspection Division and the grades are therefore official.

Protein

Total nitrogen was determined by the Kjeldahl method using mercuric oxide as catalyst. The nitrogen values were converted to protein using the factor 5.7 and corrected to the basis of 13.5% moisture.

Baking

In 1932 the simple and bromate formulas were used, while in 1933 the malt-phosphate-bromate formula (1) was added. The bromate formula included 1 mg. of potassium bromate per loaf, and the malt-phosphate-bromate included 0.3 gm. of 200 lintner malt, 0.1 gm. of ammonium dihydrogen phosphate and 1 mg. of potassium bromate per loaf.

Results

Moisture in Standing and Harvested Grain

In 1932 the moisture content of the grain in both fields was low before harvesting started. The weather was dry and warm, and all samples dried fairly rapidly (Table I). The rate of drying was most rapid in swathed grain and slowest in barge stacks, but in all cases samples were dry when threshed.

TABLE I

MOISTURE IN GRAIN, AND WEATHER CONDITIONS, 1932

Date	Moisture in grain as % of total weight				Atmospheric temperature	Relative humidity	Rainfall	Average barge stack temperature
	Standing	Swathed	Stooked	Barged				
Field 1								
Aug. 25	25.9*	25.9*	25.1*	28.4*	—	—	nil	—
27	20.4	15.8	—	24.2	68	28	nil	71
30	20.1	15.0	16.9	23.6	64	37	nil	64
31	—	—	—	—	—	—	0.01	—
Sept. 1	17.8	13.2	15.4	16.3	76	33	0.02	79
2	—	—	—	—	—	—	0.01	—
5	11.0	10.9	13.0	15.9	79	33	nil	80
7	11.0	9.6	11.5	14.2	82	—	nil	82
8	—	—	—	—	—	—	0.13	—
10	14.2	—	—	—	—	—	nil	—
12	—	11.7	12.8	13.3	75	31	nil	—
Field 2								
Sept. 5	23.6*	23.6*	24.1*	23.6*	79	33	nil	—
7	22.5	20.6	22.6	23.1	82	26	nil	83
8	16.7	12.4	—	20.2	—	—	0.13	—
10	14.5	—	15.9	17.9	82	29	nil	81
12	—	12.9	12.8	13.8	—	—	nil	—

**Average moisture content at cutting.*

In 1933 the moisture contents of grain samples were higher when harvesting started than in 1932. The weather was cooler and much damper, both because of higher humidities and more rainfall. Drying of grain was much slower except for swathed samples, and even these showed a subsequent increase as a result of a heavy rain on August 31 (Table II). The grain in the barge stacks dried more slowly than did the other lots, but was less affected by rainfall. These samples dried so slowly that two weeks after the other lots were threshed these were still tough, but it was considered inadvisable to delay threshing further. Stooked and standing grain were affected by rain, but not to the same extent as swathed material. Both dried out relatively rapidly.

TABLE II

MOISTURE IN GRAIN, AND WEATHER CONDITIONS, 1933

Date	Moisture in grain as % of total weight				Atmospheric temperature	Average relative humidity
	Standing	Swathed	Stooked	Barged		
Field 1						
Aug. 23	31.0*	31.0*	31.0*	31.0*	—	—
26	22.1	19.3	25.6	25.3	78	54
28	16.4	16.1	19.8	28.7	89	52
30	23.9	16.4	13.9	25.8	58	74
Sept. 1	23.3	23.5	25.8	25.7	57	58
3	21.3	19.4	20.5	20.7	66	58
5	20.2	21.2	19.5	20.9	40	81
7	23.8	24.2	20.9	19.9	56	68
9	18.1	17.9	17.9	21.5	83	56
11	17.6	20.8	15.7	22.7	67	46
13	14.6	16.5	14.6	17.9	73	58
Field 2						
Aug. 23		42.0*	42.0*	—	—	—
24		—	—	39.0*	—	—
26		31.7	34.5	27.8	78	54
28		16.8	23.7	31.2	89	52
30		15.5	22.0	25.5	58	74
Sept. 1		22.3	24.1	27.8	57	58
3		30.0	24.3	29.5	66	58
5		20.8	20.0	31.8	40	81
7		23.3	21.2	27.7	56	68
9		19.3	16.9	25.5	83	56
11		20.8	15.9	22.1	67	46
13		15.4	14.6	22.3	73	58

**Average moisture content at cutting.*

Temperature of Barge Stacks

Only a few readings of the temperatures in barge stacks were made in 1932 (Table I). There was little evidence of local heating or sweating, and the temperatures were close to those of the atmosphere.

Since the grain in the stacks was higher in moisture, and the weather conditions less favorable for drying in 1933 than in 1932, a more extensive study of stack temperatures was made. Individual readings are recorded in

Table III, and it is evident that there was little if any tendency for the stacks to heat. There must have been good circulation of air throughout the stacks because the stack temperatures were similar to atmospheric temperatures even when there were relatively abrupt changes in the latter from day to day. The readings taken on August 30 seem higher than the atmospheric temperature, but these represent the maximum reached during the preceding 48 hours. There was a tendency to greater variations in temperature near the tops than at the centres or bottoms of the stacks. There were only a few spots where local heating occurred, these being somewhat more frequent

TABLE III

Temperature of barge stacks, and weather conditions, 1933

Date		Hour	Moisture	Maximum atmospheric temperature, °F.	Rainfall, in.	Average relative humidity	Temperature of stacks, °F.		
							Top	Centre	Bottom
Field 1									
Aug.	24	11.45	35.2	68	nil	58	71	71	71
	25	11.20	36.7	75	nil	54	62.5	62	62
	26	10.50	25.3	78	nil	54	71	71	71
	27	10.35	25.8	80	nil	40	61	61	61
	28	10.35	28.7	89	nil	52	61	61	61
	30	16.30	25.8	58	nil	77	80	70	70
	31	11.40	22.1	56	0.66	84	60	59	58
Sept.	1	11.20	25.7	57	nil	58	57	57	57
	2	11.50	21.2	60	nil	57	51	50	50
	3	11.50	20.7	66	nil	58	47	49	50
	4	12.30	23.1	58	nil	64	57	52	49
	5	11.00	20.8	40	0.07	81	50	49	48
	6	11.30	20.4	43	0.09	43	40	41	43
	7	11.20	19.9	56	nil	68	44	44	44
	8	12.00	24.5	79	nil	62	60	60	58
	9	11.20	21.5	83	nil	56	68	64	58
	10	12.20	19.1	65	0.05	60	65	66	63
	11	11.25	22.7	67	0.04	46	59	58	57
Field 2									
Aug.	24	11.30	38.7	68	nil	58	71	71	71
	25	10.20	36.7	75	nil	54	62	62	62
	26	10.30	27.8	78	nil	54	67	66	66
	27	10.20	33.4	80	nil	40	62	61	61
	28	10.20	31.2	89	nil	52	61	61	61
	30	11.30	25.5	58	nil	74	71	70	63
	31	10.20	26.8	56	0.66	84	59	58	56
Sept.	1	11.00	27.8	57	nil	58	56	57	57
	2	11.05	28.2	60	nil	57	52	49	50
	3	11.30	29.5	66	nil	58	49	50	50
	4	12.00	25.9	58	nil	64	50	48	48
	5	10.40	31.8	40	0.07	81	49	51	49
	6	11.20	25.2	43	0.09	43	41	41	45
	7	10.50	27.6	56	nil	68	44	44	50
	8	11.30	23.4	79	nil	62	50	48	50
	9	11.10	25.5	83	nil	56	65	64	63
	10	12.00	23.7	65	0.05	60	60	63	63
	11	11.00	22.1	67	0.04	46	59	59	58

in the stacks in Field 2 than in those in Field 1. Most of the stacks in Field 1 were molded to some extent, and those in Field 2 were much worse. Sprouting of the grain took place in a layer two to six inches deep at the bottom of many stacks, and some of the grain from Field 2 had to be discarded. Sprouted grain was found near the top of a few stacks where rain had penetrated.

Temperature and Moisture in Stored Combined Wheat

The straight-combined wheat was relatively free from weed seeds when stored, and contained 13.9% moisture in 1932, and 14.0% in 1933. On the day when combining was done in 1932, the maximum temperature was 82° F., while in 1933 it was 77° F. The grain went into the bin at temperatures close to these figures, as indicated by the readings taken on the day, or the day after, the grain had been binned. Temperatures were taken twice daily for several days, and then daily. The results are recorded in Table IV. Moisture determinations were made regularly in 1933, but only once in 1932.

TABLE IV

TEMPERATURE AND MOISTURE OF STRAIGHT-COMBINED WHEAT IN THE BIN

Date	Hour	Maximum atmospheric temperature	Average moisture	Temperature of wheat, °F.		
				Top	Centre	Bottom
1932						
Sept. 10	21.50	82	13.9	—	80	79
11	10.50	63	—	—	83	76
11	20.00	63	—	—	83	79
12	8.00	66	14.5	—	82	83
12	21.00	66	—	—	83	74
13	8.00	78	—	—	81	79
13	21.30	78	—	—	81	71
14	14.00	63	—	—	82	75
14	19.30	63	—	—	81	77
15	21.00	62	—	—	82	74
16	21.05	62	—	—	81	72
17	22.00	68	—	—	80	74
20	11.00	51	—	—	81	70
21	18.00	58	—	—	78	65
24	8.30	61	—	—	78	66
1933						
Sept. 14	10.30	77	14.0	73	70	68
15	10.30	55	14.0	73	73	74
15	21.10	55	—	70	72	66
16	9.30	60	14.1	71	71	71
16	19.00	60	—	65	72	67
17	14.00	54	14.4	68	73	74
18	12.00	49	13.9	67	71	69
19	11.00	64	13.7	67	69	60
19	16.30	64	—	69	68	61
20	10.50	72	13.4	66	68	62
20	16.50	72	—	65	68	60
21	10.00	66	14.1	66	66	62
22	9.40	60	12.0(?)	64	66	57
23	14.45	50	14.0	62	65	52
24	16.00	41	14.0	59	58	58
25	17.00	39	14.1	57	60	53

In neither year's results is there indication of a significant increase in either temperature or moisture content of the stored grain. The temperatures decreased slowly, particularly in 1932 when the bulk of grain was much larger than in 1933. The very slow decrease in temperature is probably due to the slow diffusion of heat in bulked grain as discussed by Swanson and Fenton (6).

Grade and Weight per Bushel

The grades and weights per bushel of all samples at the time of threshing are recorded in Table V.

TABLE V

GRAIN CHARACTERISTICS

Method of harvesting	Date of threshing	Average moisture at threshing, %	Grade*			Weight per bushel, lb.
			At threshing	After laboratory storage	After bin storage	
1932						
Field 1						
Binder	Sept. 12	12.4	1°	—	—	65.0
Swather	Sept. 12	11.7	1°	—	—	60.5
Combine	Sept. 10	14.2	1°	—	—	62.0
Barge	Sept. 12	13.3	1 Hard	—	—	65.5
Field 2						
Binder	Sept. 12	12.7	1 Hard	—	—	65.0
Swather	Sept. 12	12.9	1°	—	—	64.5
Combine	Sept. 10	13.9	1°	—	—	64.0
Barge	Sept. 12	13.8	1 Hard	—	—	66.0
1933						
Field 1						
Binder	Sept. 13	14.6	1°	1°	—	64.5
Swather	Sept. 14	13.8	1°	1°	1°	63.0
Combine	Sept. 13	13.7	1°	2°	1°	64.0
Barge	Sept. 29	16.4	4 tough	3°	2°	60.5
Field 2						
Binder	Sept. 13	14.6	1°	1°	—	64.5
Swather	Sept. 14	14.0	1°	1°	—	63.0
Barge	Oct. 3	16.7	5 tough	4°	4°	61.5

**1° = No 1 Northern; 2° = No. 2 Northern, etc.*

In 1932, grades were all high, the two barge samples and one binder sample grading No. 1 Hard and the remainder No. 1 Northern. There were fairly wide variations in weight per bushel; the two barge samples were slightly heavier than the binder samples, which in turn were significantly heavier than either the swather or combine samples.

In 1933, there were marked variations in both grades and weights of the barged wheat as compared with other samples. The binder, swather and straight-combine samples all graded No. 1 Northern, while the barge samples graded Nos. 4 and 5 tough. The swather samples were somewhat bleached, but graded No. 1 Northern. The barge samples were dark in color, musty, and contained sprouted kernels, the grain from Field 2 being worse than that from Field 1. The barge samples were also lowest in weight per bushel, while the binder samples were heaviest.

Grades were obtained again after samples had been stored in small cans in the laboratory, and the bulk of the grain bin stored. The results show that there was a marked improvement in the grade of barged grain as a result of storage. The grade of the barged grain from Field 1 was raised from No. 4 Northern to No. 3 Northern after laboratory storage and to No. 2 Northern after bin storage. The grade of the barged grain from Field 2 was raised from No. 5 to No. 4 Northern after both laboratory and bin storage. The straight-combine sample, which had graded No. 1 Northern at threshing was graded No. 2 Northern after laboratory storage, but No. 1 Northern after bin storage.

Protein, Milling and Baking Results

In 1932, the protein content of the barge and binder samples was lower than that of those harvested by the other methods (Table VI). It is possible that this can be attributed to sampling error since this relation did not hold in 1933.

The milling results are not tabulated because there was but little difference with the various samples. The swather sample from Field 1 gave slightly the highest yield, but all yields were high.

The baking results are reported in Table VI. There are no marked differences between those obtained with the simple and those with the bromate formula. In general these results parallel the protein results, the binder samples being the poorest and the swather and straight-combine samples from Field 2 the best. The protein level of these samples is in the range where a variation of 1.2% in protein must be considered important in respect to baking quality.

As already indicated, the 1933 studies were somewhat more extensive. Small samples of each lot were collected at the time of harvesting and stored in cans in the laboratory. The barge, swather and straight-combine samples which were bin stored were sampled in March, 1934. Both lots of samples were milled and baked.

The protein results (Table VI) are more uniform than those obtained with the 1932 samples. There were apparent small increases in protein content during bin storage, which may have resulted from loss of carbohydrates by respiration, or may have been the result of experimental error only. In any case, the difference between the highest and lowest protein content is only 0.6% which is not sufficient to affect the baking results markedly, since the general protein level was higher than in 1932.

The milling yields were all lower than those obtained from the 1932 samples. The barge samples gave slightly higher yields than did the others, but the differences were hardly significant.

TABLE VI

GRADE, PROTEIN AND BAKING DATA

Method of harvesting	Time of sampling	Protein (13.5% H_2O), %	Absorption, %	Simple formula		Bromate formula		Malt-phosphate-bromate formula	
				Loaf volume, cc.	Partial baking score	Loaf volume, cc.	Partial baking score	Loaf volume, cc.	Partial baking score
1932									
Field 1									
Binder	At threshing	12.4	67	598	54	621	55	—	—
Swather	At threshing	13.6	68	684	58	670	59	—	—
Combine	At threshing	13.6	67	692	58	672	53	—	—
Field 2									
Binder	At threshing	12.8	68	618	57	593	57	—	—
Swather	At threshing	14.4	69	720	58	749	57	—	—
Combine	At threshing	14.2	69	670	60	724	57	—	—
Barge	At threshing	13.1	70	650	58	665	58	—	—
1933									
Field 1									
Binder	At threshing	14.7	64	711	50	698	50	752	46
Swather	At threshing	14.7	64	648	46	576	40	608	39
Swather	After storage	15.0	64	706	48	624	51	697	43
Combine	At threshing	15.3	66	612	48	532	46	583	43
Combine	After storage	15.2	64	580	48	606	46	600	41
Barge	At threshing	14.7	66	691	42	530	36	560	35
Barge	After storage	15.0	64	670	49	640	47	694	41
Field 2									
Binder	At threshing	15.2	64	682	48	774	50	844	49
Swather	At threshing	15.1	64	731	48	618	46	638	43
Barge	At threshing	15.2	63	628	39	579	39	567	35
Barge	After storage	15.3	64	735	47	707	50	762	45

The baking results are presented in Table VI. The results obtained with the simple formula show lower loaf volumes for the straight-combine samples after both laboratory and bin storage than for other samples, and poorer scores for the barge material sampled at the time of harvesting. The low loaf volumes of combine samples persisted when these were baked by the bromate and malt-phosphate-bromate formulas, and are apparently inherently characteristic of the material. The poor scores of barge samples stored in the laboratory may be partially the result of storage in closed cans under fairly high temperatures in the laboratory. These samples when harvested smelled musty, and this smell persisted in the samples stored in the laboratory, but disappeared from those stored in the bin.

The results of the bromate baking test indicate that the binder samples were the best of those obtained at the time of threshing, but that samples harvested by the swather and barge methods and bin stored were only slightly, if at all, inferior to the original binder samples Owing to limitation of bin space, no binder wheat was bin stored so that comparisons between the storage effects on this and other samples is not possible.

The results of the malt-phosphate-bromate test indicate that the binder samples were superior both in loaf volume and scores to the samples harvested by other methods, whether sampled at the time of threshing or after bin storage. Bin storage markedly improved the quality of barge samples, to some extent improved the swather samples, but did not improve the combine material.

Taken as a whole, these results show that the binder samples were somewhat superior to those harvested by other methods. The barge samples were tough when threshed, and made the poorest bread. The loaf volumes of straight-combine samples were consistently lower than those of binder or swather samples, and the scores consistently lower than those of binder samples. Bin storage improved the barge samples most markedly, although some of the differences noted between bin- and laboratory- stored samples may be attributed to the lack of effective aeration of the latter. Bin storage improved the swather sample somewhat, but the straight-combine sample little, if any.

Discussion

The results with the barge method obtained in the second year of this experiment are directly opposite to those obtained in the first year. The differences are due in part to differences in weather conditions and probably in part to the higher moisture content of the 1933 grain when harvested. In 1932, only 0.17 in. of rain fell during the time the barge stacks were exposed, but the grain in the stacks dried more slowly than that harvested by other methods. In 1933, from the time of cutting until September 14, when all except the barge samples were threshed, there was 0.91 in. of rain. At this time the grain in the stacks had dried insufficiently to make threshing possible, and during the two weeks which followed an additional 1.63 in. of rain fell. Thus the barge stacks were subjected to 2.54 in. of rain, and in this respect the tests were unfair to this method of harvesting. It must be emphasized, however, that since the barge samples dried more slowly than the others, it was this factor which was responsible for their exposure to extra weathering. The grain was in general free from weeds and the straw not particularly green. The higher moisture content probably had some effect on the subsequent deterioration in quality because of the longer time needed for the grain to dry.

The barge stacks were deposited on ground which was slightly damp, and the temperature and humidity conditions were in general unfavorable to drying. Some of the stacks, although built as well as the equipment would allow, leaked during the heavy rains, and the resulting wet spots undoubtedly aggravated the other conditions. The poor results with the barge material may be accounted for, then, as the total effect of damp ground, high moisture content and poor drying conditions. The immediate cause of deterioration was not heating, but rather molding and stack-staining as described by Coleman and Rothgeb (2).

Under more generally favorable drying conditions it has been observed that barge stacks, in areas where this method is used more extensively, may be subjected to as much or more rain than were those studied in 1933, without injury to grade or quality. In such cases, however, the grain was probably drier when stacked than that in this study, and the weather conditions, other than the rain itself, were undoubtedly more favorable.

It must be concluded that under suitable conditions the barge method is satisfactory in producing wheat of high grade and quality. If conditions in a district are usually more or less like those which obtained during the 1932 studies, the method will be entirely satisfactory. If, however, conditions are in general more like those which obtained in 1933, as is the case in the Edmonton district, the method cannot be recommended.

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KERNEL TEXTURE AS AN INDICATOR OF QUALITY IN HARD RED SPRING WHEATS[1]

By O. S. Aamodt[2] and J. H. Torrie[3]

Abstract

The gray wooded soils found at Fallis, Alberta, provided a satisfactory means of obtaining a differentiation in kernel texture in hard red spring wheats. Correlation studies showed that the varieties behaved more or less similarly from year to year in kernel texture, protein content and loaf volume, but not in partial baking score. Kernel texture was indicated as being a better measure of partial baking score than protein content, while the latter was the better index of loaf volume. A close relation was found between the kernel texture of the varieties grown at Fallis and both the partial baking score and loaf volume of the same varieties grown at Edmonton. In the case of protein content determined on the Fallis material no such relation was obtained. The wheat-meal fermentation test was found to be of little value in differentiating between the baking quality of hard red spring wheat varieties.

Introduction

In the production of new wheat varieties the plant breeder has three major objectives. He desires to develop new varieties which possess desirable agronomic type, resistance to the important diseases, and high milling and baking quality. For the first two objectives, satisfactory technique has been developed for the testing of small samples in early generations but, unfortunately, such is not the case for baking quality.

At present the only course open to the plant breeder is to grow a large number of strains, until he has sufficient seed of homozygous lines for a milling and baking test. The unsatisfactory aspects of this method are the enormous amount of costly routine work that it necessitates, and the considerable amount of time required, before an indication of quality can be obtained. If such a system is not followed, and only a relatively few lines are carried on until a baking test can be made, the chances of securing new varieties excelling in milling and baking quality are greatly reduced. Thus it is clearly seen that the plant breeder is in great need of some simple method by which he may secure a fairly reliable indication of the baking quality of his various selections from the small samples obtained in the early generations of a cross. Such a method would make it possible to eliminate a large number of strains in the F_3 and F_4 generations, and to continue with only those strains that would be reasonably certain to possess satisfactory quality. In later generations, the baking test could be used for the final elimination of strains unsatisfactory in quality.

In the present paper the baking results of a number of wheats of a diverse nature, grown both at Edmonton and Fallis over a period of years, are con-

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sidered in respect to protein content and kernel texture. Inter-annual and inter-station correlations have been calculated to show the relation between the several characters for the different years, and for the different environments.

Materials and Experimental Methods

The series used in these studies was composed of a number of named varieties and hybrid strains of diverse origin. The material was grown in replicated rod-row trials at Edmonton, Alberta, on black soil; and at Fallis, Alberta, on gray soil, in the years 1931, 1932 and 1933. A brief preliminary report on the general procedure followed at the University of Alberta was made in 1933 (1).

The material was studied to find out the inherent tendencies of, and the relations among, the four characters, kernel texture, protein content, loaf volume and partial baking score*. The loaf volume and partial baking score** were determined by the bromate method of baking (10). At Edmonton in the years 1931, 1932 and 1933, 61, 29 and 28 varieties, respectively, were compared by the baking test. At Fallis for the years 1932 and 1933, 26 and 12 varieties, respectively, were tested. Baking tests were not made on the 1931 samples grown at Fallis. The method of securing the sample for the baking tests was to bulk the grain from the border rows of three-row quadruplicated rod row plots.

The protein content was calculated as total nitrogen × 5.7, corrected to a 13.5% moisture basis. The material was classified for kernel texture on a scale running from 1 to 10, 1 being completely starchy and 10 completely vitreous. Fisher (8) states that with small samples, less than 100, tests of significance based upon the standard error are often very deceptive. Consequently, the significance of the correlation coefficients reported herein was judged by the method suggested by Fisher (8). A correlation was considered significant if P, the probability of exceeding the observed value through random sampling, was less than 0.05. If the P value exceeded the 5% point, the correlation coefficient is marked with one asterisk, while if it exceeds the 1% point it is marked with two asterisks.

At Edmonton a satisfactory differentiation in kernel texture is obtained only in years characterized by a higher than average rainfall and late maturity. In order to overcome the uncertainty of obtaining a differentiation in kernel texture, it was found necessary to grow the material in an environment which would bring out inherent differences in the texture of the kernel. Observations made of the wheat coming from different parts of the province showed that samples of most wheat varieties grown on the gray wooded, or podsol soils, were very starchy in texture. Consequently in 1930 a small

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***Partial baking score is a value used by members of the Associate Committee on Grain Research to express in a single figure an estimate of the absorption, general appearance, crumb color and crumb texture of the loaf. The perfect scores for the various characters are as follows: loaf shape, 5; crust color, 5; crumb color, 10; and crumb texture, 10. The partial baking score is calculated as: (absorption—60) + loaf shape + crust color + 2(crumb color) + 3(crumb texture).*

plot of land in the gray wooded soil area at Breton, Alberta, was secured for experimental purposes. The following year, owing to the difficulty of reaching Breton, the location of the experimental plots was changed to Fallis, Alberta, which is located in the gray wooded soil area fifty miles west of Edmonton. The nature and composition of the soils of this area are described by Wyatt Newton and Mather (24). The results of preliminary experiments indicated that the kernel texture of wheat grown at Fallis provides a good differential reaction for baking quality. In order to test more thoroughly the above observations, the experiments and calculations reported in this paper were made.

Literature Review

Shollenberger and Coleman, 1926 (21) report that the vitreous kernels of a wheat sample are superior in loaf volume to the starchy kernels. Coleman, Dixon and Fellows, 1927 (6) and Mangels, 1927 (14) conclude that kernel texture is of questionable value as an indication of baking strength. Hayes, Immer and Bailey, 1929 (11) found that kernel texture was neither significantly nor consistently related to loaf volume.

Significant positive correlations between protein content and loaf volume for either, or both, spring and winter wheats, have been reported by a number of investigators (4, 5, 6, 12, 13, 25, 26). Larmour, 1930 (12) concludes that the relation between these two characters in sound wheat is close enough to justify the use of the protein test as a factor in the classification of hard red spring wheats. Hayes, Immer and Bailey, 1929 (11) found, from a study of a number of wheats of a diverse nature, no significant relation between protein content and loaf volume.

A review of the literature on the relation between kernel texture and protein content shows a considerable difference of opinion. Several investigators (9, 20–23) obtained a significant relation between vitreous kernel texture and high protein content Other investigators (6, 14, 15, 18) report that kernel texture is of questionable value as an index of protein content. Newton, Cook and Malloch, (16) found some evidence of a curvilinear relation existing between vitreousness of kernel and protein content.

Experimental Results

In the varietal studies the four characters, grain texture, protein content, loaf volume and partial baking score were compared. Correlation studies were made in order to measure both the tendency of the varieties to react similarly, when grown during the different years and at the different stations, and the relation between the several characters. The significance of the correlation for any character from year to year gives an indication of its heritable nature and also an expression of its reliability as an index of quality. The degree of association among the several characters was studied primarily to find out if the relations between kernel texture, protein content, loaf volume, and partial baking score were sufficiently high to warrant their use as indices of baking quality.

TABLE I

INTER-ANNUAL CORRELATIONS

Variables correlated	Edmonton					
	1931–32		1931–33		1932–33	
	No. of samples	*r*	No. of samples	*r*	No. of samples	*r*
Protein content	35	+0.440**	22	+0.612**	28	+0.252
Kernel texture	—	—	—	—	—	—
Loaf volume	18	+0.519*	17	+0.725**	18	+0.687**
Partial baking score	18	+0.373	17	+0.213	18	+0.101

Variables correlated	Fallis					
	1931–32		1931–33		1932–33	
	No. of samples	*r*	No. of samples	*r*	No. of samples	*r*
Protein content	28	+0.422*	11	+0.699*	11	+0.559
Kernel texture	24	+0.360	11	+0.607*	11	+0.817**
Loaf volume	—	—	—	—	11	+0.537
Partial baking score	—	—	—	—	11	+0.280

**P* value exceeds 5% point. ***P* value exceeds 1% point.*

The data in Table I and Table II give the inter-annual and inter-station correlations for the four characters studied. The correlations of protein content for the comparisons made were significant between the years 1931 and 1932, and 1931 and 1933, both at Edmonton and Fallis; and between Edmonton and Fallis for the years 1931 and 1932. For kernel texture the correlations, for the comparisons studied, were significant between the years 1931 and 1933, and 1932 and 1933 at Fallis, and between Edmonton and Fallis for 1931. The other correlations, both of protein content and kernel texture, although not statistically significant, indicate the trend of relationship. The correlations for loaf volume between any two years were significant, except between 1932 and 1933 at Fallis. A non-significant negative

TABLE II

INTER-STATION CORRELATIONS (EDMONTON AND FALLIS)

Variables correlated	1931		1932		1933	
	No. of samples	*r*	No. of samples	*r*	No. of samples	*r*
Protein content	52	+0.562**	28	+0.554**	11	+0.402
Kernel texture	89	+0.382**	—	—	—	—
Loaf volume	—	—	17	−0.007	11	+0.749**
Partial baking score	—	—	17	+0.129	11	+0.719*

**P* value exceeds 5% point. ***P* value exceeds 1% point.*

correlation was obtained for loaf volume in 1932, between Edmonton and Fallis. With partial baking score the only significant correlation obtained was that between Edmonton and Fallis for 1933. These correlations indicate that, for the characters studied, with the exception of partial baking score, the varieties used behave more or less similarly for the different years at Edmonton and Fallis. The lack of correlation between the partial baking scores for the different years at Fallis and Edmonton indicates that some of the components of this character fluctuate rather widely from season to season. This indicates that for the conditions of the experiment, partial baking score in itself is not a good measure of quality. However, when it is used in conjunction with loaf volume it is of considerable value.

All possible inter-relations between the four characters studied, at both Edmonton and Fallis for the years 1931 to 1933, were determined by means of both the simple and second order partial correlation coefficients. The results are given in Table III. Partial correlations were not calculated for the years 1932 and 1933 at Edmonton, because several of the variables did not appear to be related to any of the other variables, as determined by means of the simple product-moment correlation coefficient.

TABLE III

Correlations between several indices of baking quality

Variables correlated†	Simple correlations				Variables correlated	Partial correlations			
	Edmonton		Fallis			Edmonton		Fallis	
	No. of samples	*r*	No. of samples	*r*		No. of samples	*r*	No. of samples	*r*
1931									
TP	61	+0.410**	88	+0.447**	TP.VS	61	+0.340**	—	—
TV	61	+0.439**	—	—	TV.PS	61	−0.124	—	—
TS	61	+0.666**	—	—	TS.VP	61	+0.586**	—	—
PV	61	+0.439**	—	—	PV.TS	61	+0.379**	—	—
PS	61	+0.270*	—	—	PS.TV	61	−0.220	—	—
VS	61	+0.654**	—	—	VS.TS	61	+0.571**	—	—
1932									
TP	—	—	26	+0.588**	TP.VS	—	—	26	+0.278
TV	—	—	26	+0.408*	TV.PS	—	—	26	−0.095
TS	—	—	26	+0.702**	TS.VP	—	—	26	+0.541**
PV	29	+0.405*	26	+0.737**	PV.TS	—	—	26	+0.637**
PS	29	+0.060	26	+0.608**	PS.TV	—	—	26	+0.194
VS	29	+0.076	26	+0.481*	VS.TS	—	—	26	+0.104
1933									
TP	—	—	12	+0.675*	TP.VS	—	—	12	+0.566
TV	—	—	12	+0.482	TV.PS	—	—	12	+0.079
TS	—	—	12	+0.586*	TS.VP	—	—	12	+0.327
PV	28	+0.032	12	+0.391	PV.TS	—	—	12	+0.079
PS	28	+0.233	12	+0.435	PS.TV	—	—	12	+0.014
VS	28	+0.797**	12	+0.668*	VS.TS	—	—	12	+0.541

**P value exceeds 5% point.* ***P value exceeds 1% point.*

†*T = kernel texture; P = protein content; V = loaf volume; S = partial baking score.*

The simple correlation coefficients between kernel texture with protein content, loaf volume, and partial baking score were significant in all cases, with the exception of that with loaf volume, at Fallis, in 1933. Holding loaf volume and partial baking score constant, the correlations between kernel texture and protein content are lowered in every instance, and only at Edmonton for 1931 is the correlation statistically significant. In all comparisons when protein content and partial baking score are held constant, the correlations between kernel texture and loaf volume are not significant. Partial correlations of kernel texture with partial baking score, holding protein content and loaf volume constant, are significant in two of the three comparisons made. Similarly the partial correlations of protein content with loaf volume, holding the other variables constant, are significant in two of the comparisons. Between loaf volume and partial baking score, the partial correlation is significant only at Edmonton for the year 1931. The results indicate that kernel texture gives a better indication of partial baking score than does protein content, while protein content is the better index for loaf volume.

At Edmonton in most seasons it is impossible to obtain a satisfactory differentiation in kernel texture, while at Fallis large differences in the texture of kernel between varieties are secured every season. Consequently it is of first importance to know the relation existing between Fallis kernel texture and Edmonton baking quality. The data in Table IV give the correlation coefficients of kernel texture and protein content for a number of spring wheat varieties grown at Fallis, with the loaf volume and partial baking score of the same varieties grown at Edmonton. Protein content at Fallis is shown to have no significant relation with loaf volume and partial baking score at Edmonton. For the three years of the test Fallis kernel texture, however, was strongly correlated with loaf volume at Edmonton. The correlation coefficients of Fallis kernel texture and Edmonton partial baking score were significant for 1931 and 1933, but not for 1932.

TABLE IV

INTER-STATION CORRELATIONS (EDMONTON AND FALLIS)

Edmonton	Fallis			
	Kernel texture		Protein content	
	No. of samples	*r*	No. of samples	*r*
1931 loaf volume	49	+0.323*	51	+0.196
1932 loaf volume	16	+0.699**	14	+0.284
1933 loaf volume	11	+0.920**	11	+0.498
1931 partial baking score	49	+0.301*	51	+0.233
1932 partial baking score	16	−0.184	14	−0.280
1933 partial baking score	11	+0.758**	11	+0.211

**P value exceeds 5% point.* ***P value exceeds 1% point.*

From the data presented it may be concluded that the kernel texture determined on wheat varieties grown at Fallis is a reliable index as a general measure of the baking quality determined on the same varieties grown at Edmonton. As such it is considered to be of great value in obtaining a preliminary indication of the baking quality of hybrids in the early generations. In plant breeding studies conducted at the University of Alberta, kernel texture as determined on wheat grown at Fallis is being used to a considerable extent as a preliminary method of eliminating strains of undesirable baking quality.

The method employed is to plant at Fallis a replicate of all hybrids in single five-foot rows. At harvest all the plants in a row are bulked, threshed and classified for kernel texture, as described previously. The lines or strains possessing a low texture index are discarded, while those with a high texture index and of desirable agronomic type are retained for further tests. This method is continued for several years and by the fifth or sixth generation milling and baking tests are run as a final test for quality. The great advantage of this method is that it enables the elimination in the early generations of a large number of strains of poor baking quality.

The wheat-meal fermentation time test developed by Saunders and Humphries (19), Pelshenke (17) and Cutler and Worzella (7) was tried in order that this method might be compared with baking tests and the kernel-texture test suggested in this paper. The usual procedure was followed, but much trouble was encountered in determining the "fermentation time" or end point. There was little difficulty in differentiating soft, low quality wheats from hard or bread wheats. In the latter group the results with the wheat-meal fermentation method were so variable that it appeared to be of little value to the plant breeder working with hard red spring wheats. With these wheats small pieces of dough would break off, or hang down in strings, long before there was any general deterioration of the dough ball. The difficulty in securing uniformity in molding of the dough ball was an important factor which could not be overcome and which appeared to be the cause of this irregular and variable breaking down of the dough.

There were marked differences in the baking quality of the wheat varieties grown at both Edmonton and Fallis. These varietal differences were readily demonstrated by the baking and kernel-texture tests, but not by the wheat-meal fermentation test for either the soft or hard wheat groups. As a result of these experiments it was concluded that the wheat-meal fermentation test is of little value to the plant breeder or cereal chemist, in differentiating between the quality of varieties of hard spring wheats or the quality of the same variety when the grain is produced under such extreme environmental conditions as those existing at Edmonton on the black soil, and at Fallis on the gray soil.

Discussion

The results of the present paper indicate that, for hard red spring wheat varieties grown under environmental conditions such as the gray soils, which bring out consistently the inherent differences in kernel texture, this character is of considerable value as an index of baking strength. Correlation studies with diverse wheat varieties show that kernel texture is inherited from year to year to the same extent as loaf volume and protein content. Larmour (12) from studies of protein content and loaf volume determined by both the simple and bromate methods, concludes that the relation between protein content and loaf volume (bromate) is sufficiently high to warrant the use of protein in evaluating the quality of hard red spring wheats. The data reported herein also lend support to the evidence that protein content is of value as an indication of baking quality as measured by the bromate bake.

Since both kernel texture, as determined on samples grown at Fallis, and protein content are correlated with baking quality, the use of both of these methods would give a better measurement of baking quality than either alone. Protein content can be determined accurately from small samples of grain. However, the cost prohibits its use in estimating the quality with hybrid populations, where a large number of individuals are grown. In order to secure an estimate of baking quality from kernel texture, all that is necessary is to grow the material under a differential environment and classify for texture after threshing. The data obtained from the varietal studies reported earlier in this paper indicate that kernel texture is a better indication of baking quality than is protein content. For these reasons the kernel texture of wheat grown at Fallis is a more practical index of baking quality than is protein content for hybrid populations.

Aamodt and Torrie (2), and Aamodt, Torrie and Wilson (3), by genetic studies of several series of crosses show clearly that the kernel texture of wheat grown at Fallis is a heritable character. The uncertainty of securing a differential reaction at Edmonton for this character demonstrates the necessity of growing the material in a differential environment in order to obtain data of value. The small variations in kernel texture which commonly occur in certain years in the black and brown soil areas of western Canada are of little value for the purpose of estimating baking quality. However, as indicated from this study, the gray wooded soil area of Alberta affords an excellent environment for obtaining a differential reaction for kernel texture every year.

In certain years early frosts do considerable damage to the kernel before it has had an opportunity to mature properly. This is particularly true of hybrid material grown in spaced nursery rows in the earlier segregating generations. When wheat is severely injured by frost, the ordinary baking tests are of little value in differentiating between varieties. Under these conditions the differences in texture, when the grain is grown on gray soil, are still observable and can be used to estimate differences in quality when all other methods fail.

The method of obtaining an estimate of baking quality by securing a differentiation in kernel texture is not put forward as a general substitute for the milling and baking test. However, the authors do believe that the kernel-texture index determined on samples grown at Fallis, on the gray wooded soil, is of considerable value in obtaining a preliminary indication of baking quality in the early generations of hybrid material.

Acknowledgment

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A COMPARATIVE COLOR TEST FOR COUMARIN AND MELILOTIC ACID IN *MELILOTUS* SPECIES[1]

By J. S. Clayton[2] and R. K. Larmour[3]

Abstract

The coupling reaction between phenolic bodies and diazotized *p*-nitraniline in alkaline solution is shown to be of value in the detection and estimation of the coumarin and melilotic acid content of small quantities of sweet clover. The intensity of crimson color developed on treatment of pure coumarin solutions is shown to be proportional to the concentration of coumarin and to be adaptable to colorimetric measurements. For practical purposes it is shown that this reaction can be used for estimation of coumarin and melilotic acid together.

It is possible to obtain the crimson color directly with plant extracts containing coumarin or melilotic acid, and a tentative method enabling comparisons to be made among small amounts of plant material is outlined. A comparison between results obtained by the colorimetric method outlined and by that of Obermayer shows that the latter method is open to several serious errors in the estimation of coumarin.

Introduction

The sweet clovers (*Melilotus alba* and *M. officinalis*), used as leguminous forage crops, are characterized by the presence of a "bitter principle" which affects their palatability as stock feed, and which imparts to the plant a distinct, but not unpleasant, vanilla-like odor. This property has long been associated with the presence of coumarin or its derivatives in the tissue of the plant. Individual plants appear to differ in their content of coumarin as well as in other characteristics, and attempts have been made to improve the plant by selection, particularly to secure a non-bitter variety. In 1913, Obermayer (7) stressed the need for a chemical test for coumarin in sweet clover which could be used in the selection of strains for breeding work. He submitted a procedure for the quantitative determination of coumarin which has been generally accepted as the standard method for use with plant material. This method consists in the main of distillation of the coumarin from an ether extract of the plant material and titration with standard potassium permanganate solution. It is difficult to obtain satisfactory replication of results by Obermayer's procedure and consequently a number of other methods have been proposed. Recently Duncan and Dustman (3) have shown that ether extraction of the plant is unsatisfactory, and they have proposed a modification of Obermayer's method in which the ether extraction of the original plant material is replaced by direct distillation under reduced pressure.

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Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Canada. This paper includes results of work performed by J. S. Clayton in partial fulfilment of the requirements for the degree of Master of Science at the University of Saskatchewan, and also further studies with financial assistance from the Dominion Forage Crops Laboratories, Saskatoon.

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Another method recently published is that of Kanewskaja and Fedrowa (4) whose technique is based on the separation of coumarin and melilotic acid from an ether extract of the material by converting them to their sodium salts, which are not soluble in ether. The coumarin and melilotic acid are separately recovered by acidification and subsequent ether extraction at different degrees of acidity. They are then weighed. This method is open to the criticism applied by Duncan and Dustman (3) to Obermayer's method, with regard to the unsatisfactory nature of the initial extraction with ether. In following out this method here, difficulty was found in obtaining a separation of the coumarin and melilotic acid from the plant pigments.

All these methods are slow and require large amounts of material and considerable equipment. Consequently none of them are suited to the rapid testing of single plant selections. To be of practical importance in plant-breeding work, a test for coumarin in sweet clover should be: (i) applicable to small amounts of material, so that individual plants may be tested without injury, or a small portion of the seed yield from a single plant may be analyzed; (ii) simple and inexpensive and rapid enough to permit treatment of a large number of samples daily; and (iii) capable of relatively quantitative interpretation. In this paper is presented a relatively quantitative colorimetric test for coumarin in sweet clover. It is hoped that the test will be of use to the plant breeder.

Occurrence and Combination of Coumarin Compounds in Plant Tissue

The forms in which coumarin exists in plant tissues have been the subject of much investigation. In the tonka bean, coumarin is often found in pure crystalline form between the seed cotyledons, and extraction is carried out on a commercial scale. In *Melilotus officinalis*, coumarin seems to be found usually combined with or associated with melilotic acid. Zwenger and Bodenbender (9) state that coumarin in *Melilotus* occurs in combination with melilotic acid. Later, Wischo (8) described a distillation of dried *Melilotus* from which were obtained coumarin, melilotic acid and its anhydride, and a little melilotaldehyde. In later years evidence has been given to show that these compounds occur in part in the form of glucosides. Bourquelot and Hérissey (1) obtained from *Melilotus* and *Asperula* glucosides which on hydrolysis with emulsin yielded coumarin. They obtained a larger percentage extraction of coumarin from an acid hydrolysis than from a boiling aqueous extract. Navez (6) isolated from *Melilotus albus* and *M. altissimus* a substance which he called coumarigenin, and which appeared to be a glucoside yielding coumarin hydrocoumarate and *d*-glucose on hydrolysis. Thus, coumarin is to be found in plant tissue with other associated compounds of the general type of hydroxyphenylpropionic or hydroxycinnamic acid.

Melilotic acid, which can be prepared readily by reduction of coumarin, has a peculiar honey-like odor, typical of sweet clover, and, although sharply acid in taste, is not as bitter as pure coumarin. By distillation of melilotic acid is obtained an oily substance which is quite bitter and similar in taste to coumarin. On hydrolysis this substance is converted to melilotic acid. It is open to question whether the bitter taste of sweet clover is attributable to coumarin alone or whether it is caused by both coumarin and melilotic acid. Though the exact relation between these substances in the plant is not known, it is evident that they usually occur together, and therefore a chemical test that would indicate the presence of both these substances might be just as useful from the plant breeder's standpoint as a determination of coumarin alone.

The Azo Coumaric Acid Color Reaction

It seemed evident from the foregoing considerations that a delicate color reaction, capable of application to small amounts of plant extract, would be highly desirable, and to this end the possibilities of the coupling reaction with coumarin giving azo dyes or colors was investigated. Chakravarti (2) in 1931 reported the preparation of dyes by the coupling of diazotized arylamines with coumarin in sodium hydroxide solution, and stated that the majority of dyes so formed are yellow or red. Accordingly, solutions of various arylamines were diazotized and treated with coumarin in alkaline solution and the colors observed. It was decided to use *p*-nitraniline in this work because the color reaction appeared to be very sensitive. A distinct crimson color was obtained with concentrations of pure coumarin as low as 1 : 125,000, and the color is detectable in concentrations of less than 1 : 1,000,000.

The crimson color is developed only after the addition of alkali and evidently depends on the breaking of the lactone structure of the coumarin to give a free phenolic hydroxyl group. After the color has been developed, addition of acid turns the solution yellow, and if the solution is concentrated enough, gives a precipitate of an orange colored compound. The crimson color is not removed from an alkaline solution by ether, but when the solution is acidified the compound dissolves in the ether layer. The point of change from red to yellow is quite sharp, and the substance may be said to have indicator properties. Prolonged boiling of the solution does not destroy, but intensifies the color. On cooling the solution, the original intensity of the color is restored.

The Applicability of the Reaction for Coumarin Detection in *Melilotus* Species

Owing to the extreme sensitivity of the test, it was thought that it would prove an excellent one for identification of coumarin in sweet clover if similar results could be obtained with a plant extract. A number of extracts of sweet clover, aqueous, alcoholic and ethereal were treated with the reagents and all developed a crimson color which was not appreciably masked by the green color of the extract. It was also found that an extract made from a single leaf of the plant gave a distinct crimson color, and that a direct staining test could be obtained on a cross section of a stem of sweet clover. The question at once arose "Is this red color due to coumarin only or to other compounds likely to be present in the plant extract?"

Melilotic acid is very frequently associated with coumarin and on account of the close similarity in the structure of these two substances, they should behave similarly in respect to this reaction. Furthermore, any other phenolic or arylamine derivatives, if present, might be expected to couple and produce color. However, the number of such substances likely to be found in the extracts of sweet clover is limited and, as will be shown later, it is improbable that any except melilotic acid, coumarin and closely related derivatives are to be found in appreciable quantity in this plant.

Experimental

Specificity of Test

A number of aryl compounds were treated with the coupling reagents and any showing a red color were tested further in a more dilute solution (1 : 125,000). Of the substances tested, saligenin, guaicol, phenol, coumarin and melilotic acid gave distinct red colors at this concentration, but tyrosine gave only a faint red. However, if phenol, tyrosine or any other interfering substance is present in the sweet clover extracts, one would expect them to be present in some or all of the other grasses and legumes similar to sweet clover. Thus, if a number of other plant materials are extracted and tested with the diazotized solution and a red color is not produced, it would be reasonable to conclude that a red color produced in sweet clover is due to coumarin or melilotic acid and not to any other compound. Results given in Table I substantiate this conclusion.

TABLE I

COLORS OBTAINED BY TREATING ALCOHOLIC EXTRACTS OF VARIOUS PLANTS WITH STANDARD DIAZONIUM SOLUTION IN ALKALI

Alcoholic extracts tested	Color obtained
Brome grass	Pale yellow to green
Trigonella	Pale yellow to green
Oats	Pale yellow to brown
Western rye	Pale yellow to brown
Reed canary grass	Pale yellow to brown
Sweet grass	Pale yellow to brown
Couch grass	Pale yellow to brown
Barley	Pale brown to green
Wheat	Pale brown to green
Alfalfa no. 1	Pale brownish yellow to olive
Alfalfa no. 2	Pale brownish yellow to olive
Crested wheat grass	Pale light brown
Alpha sweet clover	Brilliant crimson red
Redfield yellow sweet clover	Deep crimson red
Arctic sweet clover	Deep crimson red
Albatrea sweet clover	Deep crimson red
Alfalfa with 0.0002 gm. of coumarin added	Deep crimson red

It can be seen from Table I that the diazonium solution gave no indication of crimson coloration except in the case of the *Melilotus* species, and in the extract to which pure coumarin had been added. With these exceptions, the extracts tested gave pale colorations ranging from olive green to reddish brown. Undoubtedly this shows that coupling by some unknown compounds is taking place in all these extracts, but that these can be present in only small amounts. The addition of small amounts of coumarin to any one of these extracts is sufficient to give a distinctive red color to the solution when treated. Thus, it is comparatively safe to assume that when the test is applied to extracts of *Melilotus* species, the development of a distinctive red or crimson color is indicative of the presence of coumarin and melilotic acid or their simple derivatives. A number of extracts of seeds of various grasses gave similar results. Only in those of *Melilotus* species was there any indication of a crimson color.

STANDARDIZATION OF THE TEST

It was decided to investigate the possibilities of developing a quantitative colorimetric test for coumarin and melilotic acid in sweet clover. A number of colorimetric determinations of organic compounds by the use of diazonium salts have been devised in late years. Koessler and Hanke (5) in the course of their study on reactions of *p*-diazobenzene sulphonic acid standardized a method of diazotizing suitable for such work. The technique outlined in this paper for diazotizing is largely based on their method, with minor modifications.

In order to test small quantities of material, it was necessary to have conditions suitable for obtaining a maximum color with 0.001 gm. of coumarin or less. Since the reaction is a coupling one, the proportion of the diazonium compound used must be sufficient to react with the maximum amount of coumarin likely to be present in the extract. Secondly, as toward the neutral point the color changes from red in base to yellow in acid, it is necessary to have an excess of alkali for each test. Too great an excess of alkali, however, causes a decrease in the intensity of the color. For all colorimetric comparisons the final volumes of the test solutions must of necessity be equal. The final volume can be varied to suit the quantity of material tested. For colorimetric comparison the solution should be relatively dilute, for the colors produced are too intense in concentrated solution for accurate matching.

The procedure was as follows. To not more than 40 cc. of the solution to be tested for coumarin, 2.5 cc. of 1.1% sodium carbonate solution was added and the liquid was heated nearly to boiling on a water bath, in order to permit equilibrium between the lactone and acid configuration of the coumarin molecule to be speedily reached, and allowed to cool slowly. To this, 5 cc. of the diazonium solution* was added and the volume made up to 50 cc. with distilled water.

The solutions were allowed to stand for 30 min. at room temperature before comparing the colors to permit equilibrium to be established.

**Diazonium solution*

Solution A—*p-Nitraniline hydrochloride.*

p-Nitraniline (3.5 gm.) was dissolved in 45 cc. of 37% hydrochloric acid and the solution was diluted to 500 cc. with distilled water, and filtered. The solution will keep indefinitely if stoppered.

Solution B—*Sodium nitrite*

Sodium nitrite (5 gm.) was dissolved in 100 cc. of distilled water. This solution is not as stable as Solution A and should be renewed frequently. It is best to keep the solution away from the light and in a dark bottle.

The diazonium solution, which the authors have found convenient to make up in 100 cc. lots, was prepared as follows: Stock solutions A and B were placed on ice before preparation of the diazonium solution. Solution A (3 cc.) and Solution B (3 cc.) were placed in a 100 cc. volumetric flask which was immersed in an ice bath for five minutes. To this, 12 cc. of Solution B was added, the flask shaken and returned to the ice bath for an additional five minutes. The flask was then filled to the 100 cc. mark with ice-cold, distilled water and kept on ice for 15 min. before using. If kept on ice, the diazonium solution will remain stable for at least 24 hr.

QUANTITATIVE NATURE OF THE COLOR TEST

In a number of preliminary experiments with solutions of pure coumarin it was observed that the intensity of the crimson color appeared to be proportional to the concentration of coumarin, and after a standard procedure had been developed for the preparation of the diazonium solution, a series of quantitative comparisons was made on coumarin and on melilotic acid, and these were compared with results obtained with Obermayer's test.

To ascertain whether the colors developed with coumarin were proportional to the concentrations a series of solutions of known coumarin content was made up and treated in the prescribed manner. The colors developed were matched against one another in a standard colorimeter. The results given in Table II show that, over a wide range of concentrations, under the conditions of the test the intensity of color is proportional to the concentration.

TABLE II

COLORIMETRIC MEASUREMENTS OF COUMARIN CONCENTRATION

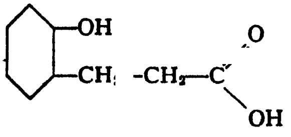
Initial concentration of coumarin, mg./38.5 cc.	0.20	0.30	0.40	0.50	0.60*	0.70	0.80	0.90	1.0
Calcd. concn. from colorimetric measurements, mg./38.5 cc.	0.20	0.30	0.42	0.53	0.60*	0.71	0.80	0.89	0.99

NOTE:—*The coumarin solution was prepared so that 1 cc. of solution was equivalent to 0.2 mg. of coumarin. (The final solutions in each case were diluted to 38.5 cc. instead of 50 cc. as presently recommended.)*

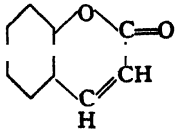
* *Used as standard.*

These and other similar results show the possibility of determining coumarin quantitatively by the colorimetric method. However, it can be seen that in testing sweet clover, a difficulty arises owing to the likelihood that melilotic acid is present as well as coumarin. If this were so, the intensity of the crimson color would be proportional to both. However, in considering the test from the standpoint of the plant breeder, a combined determination would present no handicap, since evidently these substances occur together and are the cause of the bitter taste which is so undesirable.

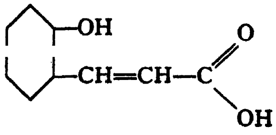
From an inspection of the formulas of melilotic acid (I, mol. wt. 166.08), coumarin (II, mol. wt. 146.05) and coumaric acid (III, mol. wt. 164.06), it is seen that there is very little difference in molecular weight and comparatively little difference in organic structure, the coumarin being unsaturated and the melilotic acid saturated.



(I)



(II)



(III)

As the development of the crimson color with the diazonium solution is primarily due to the coupling with the hydroxyphenyl grouping, it would be expected that the color developed by a given quantity of coumarin would be about the same as that developed by a similar concentration of melilotic acid, and that for practical purposes these substances could be determined together. This was shown to be so in the following experiment.

A solution of coumarin and a solution of melilotic acid were made up so that 1 cc. of each was equivalent to 0.0002 gm. of reagent. A number of test tubes were set up containing definite amounts of coumarin and melilotic acid, and also some with mixtures of the two. These were treated and compared, one against another, and within the limit of error of the readings of the colorimeter these appeared to coincide with a mean ratio of coumarin : melilotic acid : : 1 : 1.012. On the basis of the molecular weights of coumarin and melilotic acid the ratio should be 1 : 1.137 and on the basis of coumaric acid to melilotic acid 1 : 1.0123. It can be seen that whereas coumarin does not develop the crimson color to the same extent as melilotic acid on a molecule-to-molecule basis, yet for practical purposes the results obtained by comparing a mixture of coumarin and melilotic acid with a standard coumarin solution can be interpreted as the concentration of the coumarin–melilotic-acid mixture. Further work in separating the coumarin from the melilotic acid might profitably be done. The work of Kanewskaja and Fedrowa (4) in this respect should be followed up.

COMPARISON OF OBERMAYER AND COLORIMETRIC TESTS

Pure Coumarin

As the method of Obermayer has been accepted as a standard procedure for coumarin determination, a comparison of the two methods was made. This was first done in the case of pure coumarin solutions.

Pure coumarin (0.1 gm.) was distilled from a calcium chloride solution, as in the Obermayer procedure. Aliquot portions were tested for coumarin by Obermayer's titration and also by colorimetric comparison with standard coumarin solutions. The results are shown in Table III.

TABLE III

COMPARISION OF COLORIMETRIC AND VOLUMETRIC DETERMINATIONS OF COUMARIN

Weight of coumarin originally used, gm.	0.1000
Weight of coumarin found by Obermayer titration on distillate, gm.	0.0973
Weight of coumarin found by colorimetric comparisons on distillate, gm.	0.0978

These results show that the intensity of the crimson color produced is virtually proportional to the concentration of coumarin present in the solution, and that the color test is quantitative.

COUMARIN IN SWEET CLOVER

The method was next applied to the determination of the coumarin in sweet clover, and a comparison was made with results obtained by Obermayer's method. By using the Obermayer distillation procedure it is possible to obtain from an ether extract a colorless distillate in which the crimson color can be developed nicely. However, this would entail as much time and equipment as the Obermayer method itself, and would be open to the same criticism. On the other hand, when attempts are made to produce the color directly in an alcoholic or aqueous plant extract, solutions of various plant pigments to some extent mask the true crimson color of the azo dye. However, by using the yellow-green extract of a non coumarin-bearing plant and by adding to it known quantities of coumarin, it is possible to set up a series of colors whereby a comparison of sweet clover extracts can be made. A tentative procedure adopted for this estimation was as follows: 1 gm. samples of air-dried, ground leaves and stems of sweet clover, and either alfalfa or some other non coumarin-bearing plant were heated just to the boiling point with 25 cc. of 95% alcohol. The solutions were shaken for half an hour, made up to 50 cc. with water and shaken for another half hour. The plant material was allowed to settle for one hour. Then 5 cc. of each solution was treated with the color reagents in the prescribed manner and made up to 50 cc. with water. To the alfalfa extracts there was added enough standard coumarin solution to set up a series of standards containing from 0.0001 to 0.001 gm. of coumarin. The colors developed in the sweet clover were matched against the alfalfa standards and the results expressed as percentages of coumarin. As stated before, this would include melilotic acid if present.

Duncan and Dustman (3) have shown that the ether extraction method for obtaining coumarin from sweet clover is open to error, owing to the incomplete extraction of the coumarin, and they advocate in place of this method steam distillation of the plant material at 140 mm. pressure, to be followed by the Obermayer permanganate titration. This method, though not as long as the Obermayer ether extraction procedure, is technically difficult, and is open to the same sources of error as the permanganate titration, namely, that any other substance which distils over and which is oxidizable by potassium permanganate will affect the titration. Obermayer recognized this difficulty, but he showed that the amount of impurity in the distillate from the ether extraction was small and could be neglected. According to Obermayer the ether extraction method is less likely to introduce impurities in the distillate than is direct steam distillation of the plant material. Experiment showed, however, that a considerable amount of insoluble waxy material distils over from the ether residue, and he recommended filtering it off. However, with the direct steam distillation of the plant material, Duncan and Dustman advocate removing the impurities from the distillate with lead acetate, but they state that plant materials containing only small amounts of coumarin and considerable amounts of volatile reducing substances not precipitated by lead acetate cannot be determined accurately by this method.

Another peculiarity in Obermayer's method and one which he mentions himself is brought out in a consideration of the melilotic acid in the sweet clover. This presumably would be extracted by the ether and when the ether residue was distilled should appear in the distillate as melilotic anhydride, which has a melting point of 25° C. Obermayer states that the crystalline material recovered by an ether extraction of the distillate he obtained had a melting point of 67–67.5° C., and he concludes that there could have been no other compound of coumaric acid except coumarin in the material he investigated. This appears at variance with the results of other workers who have shown that melilotic acid is present in sweet clover.

In order to investigate this point and to ascertain whether melilotic acid would be carried over in the steam distillate and oxidized by permanganate the following experiment was performed: 0.1 gm. of melilotic acid was weighed in a distillation flask and the regular Obermayer distillation was carried out. After about half an hour of distillation considerable foaming occurred and the contents of the flask became yellow to golden yellow. It was impossible to distil over as much liquid as when distilling coumarin, but when about 50 cc. was left and allowed to cool in the flask, it solidified to a glassy, brown solid, which partly dissolved when water was added, leaving a brown oily substance in the solution. The distillate was made up to 500 cc. and titrated in 25-cc. portions. On the basis of a coumarin titration each sample should have given a titration value of about 12.7–12.9 cc. of $0.1N$ potassium permanganate. Instead there was obtained a titre of 0.8 cc., thus indicating that only a small portion of the organic matter had passed over into the distillate. This distillate gave a red color with the diazonium reagent. The residue in the flask after addition of water was extracted with ether. The ether was colorless and gave no appreciable residue on evaporation. When, however, the aqueous liquid was made acid with concentrated hydrochloric acid, the color of the solution changed to a lighter brown; on extraction with ether the colored material dissolved in the ether layer. After evaporating the ether, a golden to brownish bitter oil was left. It did not have the sweetish honey-like odor of the crystalline melilotic acid. When the oil was dissolved in sodium carbonate the crimson color developed on the addition of the diazonium reagent. It was concluded that this was due to the presence of melilotic anhydride. Thus it is seen that if melilotic acid were present in the ether extract of sweet clover and the distillation carried out, a certain amount would be liable to distil over, and would interfere with the titration value for pure coumarin.

Samples (10 gm.) of air-dried sweet clover were extracted for 20 hr. with ether and subjected to Obermayer's distillation and titration, and also to colorimetric estimation. It was found that even after 20 hr. of extraction the odor of coumarin persisted in the residue. To estimate how much coumarin remained unextracted, colorimetric determinations were made on alcoholic extracts of the residue. Coumarin estimation by the colorimetric method was also made on the original plant material by comparing with a standard of coumarin in an alcoholic extract of western rye grass. The colorimetric estimations on the distillates were compared with pure coumarin standards. The mean results are shown in Table IV.

TABLE IV

COMPARISON OF OBERMAYER'S AND THE PROPOSED COLORIMETRIC DETERMINATION OF COUMARIN IN SWEET CLOVER

Material tested	Method used	Coumarin, %
1. Total plant material	Colorimetric on alcoholic extract	0.3103
2. Residue from ether extraction	Colorimetric on alcoholic extract	0.2014
3. Distillate from ether extraction	Colorimetric method	0.0965
4. Distillate from ether extraction	Obermayer permanganate titration	0.1856
	Addition of results of Methods 2 and 3	0.2979
	Subtraction of result of 2 from that of 1	0.1089

These results show that: (i) a considerable portion of the coumarin or melilotic acid was not extracted by the ether in 20 hr.; (ii) the Obermayer distillate contains substances that are reduced by permanganate and yet do not give the color produced by pure coumarin, *i.e.*, only a portion of the titration is accounted for by the oxidation of coumarin; (iii) there is close agreement between the amount of coumarin determined by the colorimetric method in the total plant material (0.3103%) and the sum (0.2979) of the amounts found in the distillate (0.0965%) and in the residue (0.2014%).

To an ether extract of the same sweet clover sample, 0.03 gm. of coumarin was added, the ether evaporated, and the mixture distilled. By the addition of 0.03 gm. of coumarin the percentage determinations should each be raised by 0.3%. The results (obtained both colorimetrically and by permanganate titration) are given in Table V.

TABLE V

VERIFICATION OF METHODS BY ADDITION OF PURE COUMARIN TO THE ETHER EXTRACT

Method	Coumarin, %		
	Observed	Previous determination	Increase in coumarin
By titration	0.4871	0.1856	0.3105
By colorimetric method	0.3935	0.0965	0.2970

It is seen that the added coumarin appears in both determinations. It appears therefore that the extra 0.0891% obtained by permanganate titration represents other substances which reduce the permanganate and give a fictitious value for the coumarin determination.

The foregoing observations lead to the conclusion that the colorimetric method for estimating coumarin and melilotic acid is reasonably accurate, and is not subject to some of the errors which occur in the Obermayer method. The colorimetric method is very much shorter and allows a large number of samples to be tested in a short time. The tentative outline of the test calls for an extraction of 1 gm. of material, but in practice the method can be applied to smaller amounts of green material and even to short sections of leaf or stem from a single plant. By application of this method, single growing plants may, without injury, be tested for selection purposes.

APPLICATION OF COLORIMETRIC TEST TO SWEET CLOVER SEED

The alcoholic extract of the seed is of a pale lemon color, which on addition of sodium carbonate turns a bright yellow. When the diazonium solution is added a brilliant crimson results if coumarin or melilotic acid is present. In this case also it was necessary to compare the colors obtained with standard coumarin solutions in an alfalfa seed extract or an extract of some other similar seed which contains no coumarin. As a tentative procedure it is suggested that a 1 gm. sample of the seed be crushed or ground and extracted by heating it to boiling with 25 cc. of 95% alcohol, the solution shaken for half an hour, water added to make up to 50 cc. and the solution again shaken for half an hour. After allowing the solution to stand for one hour, an aliquot of 5 cc. can be tested by the usual method. A series of standards of 5 cc. of alfalfa extract prepared in the same manner should have sufficient coumarin solution added to them (from 0.0001 to 0.0015 gm.) to give a range of concentrations from 0.1 to 1.5%. Then by matching the color developed in the sweet clover extract to the nearest alfalfa standard by eye, and making the final comparison in the colorimeter, closely replicable estimates can be made. Because of the brilliant golden color of the seed extract after treatment with sodium carbonate, it is not possible to match the crimson colors over a wide range in the colorimeter, and therefore a large number of alfalfa standards should be set up.

Conclusion

(i) With a pure coumarin solution the intensity of the colors obtained by coupling with diazotized *p*-nitraniline in alkaline solution under standard prescribed conditions is proportional to the concentrations of coumarin and can be matched in a colorimeter. (ii) With a pure coumarin solution the results obtained by colorimetric comparisons agree with the results obtained by Obermayer's permanganate titration method. (iii) If melilotic acid is present in the material used for Obermayer's distillation method, reliable quantitative determinations for coumarin cannot be made, owing to a partial distillation of the melilotic acid, which is reduced by permanganate. (iv) For practical work, the color produced by treating coumarin with the diazonium solutions is roughly equivalent to that obtained with the same amount of melilotic acid, and thus a combined determination of the coumarin and melilotic acid in sweet clover can be made. (v) In testing Obermayer's method for coumarin in sweet clover and checking it with the colorimetric method it was shown that— (a) an ether extraction of sweet clover material is not complete in 20 hr.; (b) the distillate obtained in the Obermayer method contains a substance which is oxidized by permanganate, but which will not give the crimson color due to coumarin or melilotic acid on treatment with the diazonium reagents, and therefore may be regarded as impurity in the distillate.

A tentative method is outlined for a coumarin–melilotic-acid determination on alcoholic extracts of sweet clover plants by comparison with standards set up with pure coumarin in alcoholic extracts of western rye or alfalfa plant material. There is not yet sufficient proof to justify a claim that this procedure is anything but comparative, but knowing that with colorless solutions the method of coumarin determination is quantitative, sufficient reliance may be placed on the results obtained to make the test of practical importance to the plant breeder.

Further work should aim at the technical improvement of the method of extracting the coumarin from the plant material, with consequent improvement in the quantitative relations of this test for coumarin estimation.

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DECOMPOSITION AND MOVEMENT OF HERBICIDES IN SOILS, AND EFFECTS ON SOIL MICROBIOLOGICAL ACTIVITY AND SUBSEQUENT CROP GROWTH. PART II[1]

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Abstract

Further experiments at Edmonton to determine the effects on soils and on subsequent crop growth of copper sulphate, sodium chlorate, and sodium dichromate, are reported, together with new experiments with ammonium thiocyanate. The field plot experiments were limited to Edmonton black soil, but three typical Alberta soils, including Edmonton soil, were used in the laboratory experiments. Copper sulphate applied to a series of fallow plots in 1931 did not affect the yields of wheat on these plots significantly in 1932, or the yields of wheat, oats, rye and flax on these plots in 1933. The soil was not appreciably injured, even temporarily, by the copper sulphate. Sodium chlorate was applied to one series of fallow plots in 1930, and to another series in 1931. The effect of the heaviest applications (1300 lb. per acre) lasted for three years in one series, but serious injury to crops from such heavy applications did not last for more than two years in either series. Sodium dichromate was applied to a series of fallow plots and a series of wheat plots in 1932. It reduced the wheat yields very much in 1932, but did not reduce crop yields the following year in either series, as it decomposed and lost its toxicity in the soil rather quickly. Ammonium thiocyanate was applied to a series of fallow plots and a series of wheat plots in 1932, and it reduced the wheat yields even more than sodium dichromate in 1932. It retarded nitrification and did not decompose and lose its toxicity completely during the season of application, but even in the cases of the heavier applications (650 and 1300 lb. per acre) the toxic effect disappeared early in the following season. The total and straw yields of crops sown on these plots were generally increased by the lighter applications (160 and 325 lb. per acre) in 1933, but the grain yields were generally reduced by the heavier applications (650 and 1300 lb. per acre). Growth of certain annual weeds was considerably stimulated by this nitrogenous weed killer in 1933 and 1934, in the plots to which the heavier applications had been made. Laboratory experiments showed that the thiocyanate may be leached out of a soil with water; that it decomposes fairly rapidly in soils under favorable conditions of moisture and temperature and more rapidly in fertile soil rich in organic matter than in poorer soil; and that nitrification in soils is depressed for a time by the ammonium thiocyanate.

In Part I of these studies (3) experiments were reported concerning the residual effects of sulphuric acid, copper sulphate, sodium chlorate, barium chlorate and sodium dichromate applied as weed killers to certain Alberta soils. The present paper deals with further experiments with sodium chlorate, copper sulphate, sodium dichromate, and experiments with ammonium thiocyanate.

The field plot experiments were limited to Edmonton black soil, but for pot culture and other laboratory experiments the following three types of Alberta soil were generally used: Edmonton black, a fertile black loam, high in organic matter; Breton gray, a typical leached wooded soil; and Winterburn fine sandy loam, a soil of intermediate fertility. The field experiments were

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conducted on Belgravia field by permission of the Field Crops Department, University of Alberta. The general plan of the field plot experiments was shown in the publication previously referred to (3).

As a rule three concentrations of each chemical were used in the experiments. These cover the range used in practice fairly well. The concentrations are designated in this paper as Check or Untreated, and Concentrations A, B, and C. Table I shows the concentrations of the various chemicals used.

TABLE I

RATES OF APPLICATION OF CHEMICALS TO SOIL REPRESENTED BY TREATMENTS A, B, C, ETC.

	Check	A−		A		B		C		C+	
		Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre	Gm. per 100 gm.	Lb. per acre
H_2SO_4	None	—	—	.0024	30	.004	50	.008	100	—	—
$CuSO_4$	None	—	—	.0012	15	.0024	30	.0048	60	—	—
$NaClO_3$	None	—	—	.026	327	.052	654	.104	1308	—	—
$Na_2Cr_2O_7$	None	.013	163	.026	327	.052	654	.104	1308	.146	1838
$Ba(ClO_3)_2$	None	—	—	.039	489	.078	978	.156	1960	—	—
NH_4CNS	None	.013	163	.026	327	.052	654	.104	1308	—	—

Methods

Laboratory methods used in these experiments were described or referred to in Part I of these studies (3). Methods of making the following determinations were then given: Chlorate in soil extracts; nitrate in soil extracts in the presence or absence of chlorate; dichromate in soil extracts; carbon dioxide evolution in soil; bacteria and fungi in soil.

The method of determining thiocyanate in water extractions of soils treated with ammonium thiocyanate was not described in the previous publication. It is a modification of a method given by Treadwell and Hall (10, p. 601) for the determination of silver, as follows: Titrate 50 cc. portions of a one-to-five water extract of soil with standard silver nitrate (approximately 0.025 *N*) past the end point, using ferric ammonium alum, containing enough nitric acid to discharge the brown color of the salt, as the indicator. Then titrate back with standard ammonium thiocyanate to the red end point due to formation of ferric thiocyanate.

Results

SODIUM CHLORATE

Field plot crop yields showing residual effects of different applications of sodium chlorate are shown in Table II. The 1931 yields were given in Part I of these studies (3), but are included in this paper for direct comparison with the subsequent yields of these plots. Each yield represents the average of quadruplicate plots. The sodium chlorate was applied in July, 1930, and the wheat crop yields of the three following years are shown. It will be

TABLE II

EDMONTON FIELD PLOT CROP YIELDS, SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF SODIUM CHLORATE

$NaClO_3$ applied July, 1930		Wheat yield 1931	Wheat yield 1932	Wheat yield 1933
Check	Straw per acre, lb.	7760	2451	2345
	Grain per acre, bu.	50.1	26.1	17.1
A. Conc.	Straw per acre, lb.	1760	3301	2438
	Grain per acre, bu.	7.3	31.3	22.0
B. Conc.	Straw per acre, lb.	1020	2373	2399
	Grain per acre, bu.	1.7	24.3	21.8
C. Conc.	Straw per acre, lb.	340	2084	2465
	Grain per acre, bu.	0.02	20.2	20.4

observed that in 1931 growth was almost completely prevented by the largest application and greatly reduced by even the smallest application. In 1932 the wheat yields were not appreciably reduced except probably in the case of the C concentration (heaviest application) plots. In 1933 there was no reduction in yields of treated plots.

Another similar series of field plots was treated with sodium chlorate in the latter part of June, 1931; strips of wheat, oats, barley, and flax were sown across these plots in 1932, and wheat, oats, barley, rye, and flax in 1933. The yields are shown in Table III. It is apparent that the wheat yields were not reduced nearly as much the year following application of chlorate as in the case of the experiment started a year earlier. This is attributed mainly to a much higher rainfall during the late summer of 1931 than during the late summer of 1930, for, as pointed out in Part I of these studies (3) chlorates are readily leached down deeply into this soil by heavy

TABLE III

EDMONTON FIELD PLOT CROP YIELDS, SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF SODIUM CHLORATE

$NaClO_3$ applied June, 1931		Wheat yield		Oat yield		Barley yield		Rye yield	Flax yield	
		1932	1933	1932	1933	1932	1933	1933	1932	1933
Check	Straw per acre, lb.	4850	3744	6362	3026	5728	3131	3305	5186	2993
	Grain per acre, bu.	43.3	39.5	104.5	60.7	72.0	45.8	30.2	6.1	4.9
A. Conc.	Straw per acre, lb.	4330	3494	4588	2572	3160	2998	3464	3724	2572
	Grain per acre, bu.	39.6	37.5	90.9	59.2	30.6	35.8	38.1	13.4	7.9
B. Conc.	Straw per acre, lb.	2925	3025	4257	2132	2208	2305	2838	3368	2644
	Grain per acre, bu.	25.2	32.9	84.4	44.3	19.7	28.6	28.1	13.4	5.6
C. Conc.	Straw per acre, lb.	2497	2865	3347	1800	1136	2185	1745	2464	2513
	Grain per acre, bu.	23.2	31.1	62.3	42.3	10.4	26.2	12.8	13.3	5.1

rainfall. The total yields of oats, barley and flax were all decreased on the treated plots in 1932, the decreases being roughly proportional to the concentration of applied chlorate. In the case of flax, however, the heavier growth of straw on the untreated plots was accompanied by a smaller grain yield. In 1933, it will be observed, the wheat yields were still somewhat reduced by the heavier applications of 1931. The yields of oats, barley, and rye from the treated plots were decreased in 1933 (except in the one case of the rye plots which received the smallest applications of chlorate), the decrease being roughly proportional to the concentration of applied chlorate. In the case of flax, however, the differences between treated and untreated plots were not significant. It is possible, in this case, that the chlorate had leached down below the reach of the shallower rooted flax plants. Wheat alone was planted on these plots in 1934, but, owing to an outbreak of loose smut it was found necessary to plow down the crop about the middle of July. At this time there was no apparent effect of chlorate, but it is interesting to note that in the latter part of June the wheat leaves of the heaviest application plots were slightly burned, thus showing that some effect of the chlorate applied in 1931 was carried over into 1934. Before the middle of July this effect had disappeared, and it is unlikely that yields would have been appreciably reduced by the chlorate in 1934.

COPPER SULPHATE

Copper sulphate has been used successfully for the destruction of annual weeds. In Part I of these studies (3) its effect when applied to a growing crop of wheat is shown, and it was observed that soil nitrification is but slightly affected by its application.

A series of fallow plots was treated with copper sulphate on June 26, 1931. Yields of wheat in 1932, and yields of wheat, oats, barley, rye, and flax grown on these plots in 1933 are given in Table IV. Following the application of copper sulphate, in 1931, there was evidently no significant decrease in crop

TABLE IV

EDMONTON FIELD PLOT CROP YIELDS SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF COPPER SULPHATE

$CuSO_4$ applied June 26, 1931		Wheat yield 1932	Yields 1933				
			Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	4015	3345	3558	2905	3411	3838
	Grain per acre, bu.	35.5	30.6	77.2	40.2	35.7	4.8
A. Conc.	Straw per acre, lb.	4086	3225	3358	3225	3798	3691
	Grain per acre, bu.	34.2	28.0	76.2	46.3	34.5	5.5
B Conc.	Straw per acre, lb.	4013	3558	3478	3012	3691	3497
	Grain per acre, bu.	36.9	32.9	76.7	44.4	31.4	5.9
C Conc.	Straw per acre, lb.	3577	3651	3571	2932	3691	3561
	Grain per acre, bu.	28.9	30.2	81.1	41.6	31.2	4.5

yield in 1932 or 1933. The decrease of wheat yield in the 1932 C concentration plots was probably not significant, as the B concentration plots gave a somewhat higher yield of grain than the untreated plots.

SODIUM DICHROMATE

Sodium dichromate has been used to some extent as a chemical weed killer. Water or nutrient solution experiments showed, as previously reported (3), that when placed in contact with germinating wheat seeds, and in contact with growing wheat plant roots, sodium dichromate is much more toxic to wheat than sodium chlorate in equal concentration. However, it was shown, in laboratory and greenhouse experiments, that sodium dichromate decomposes and loses its toxicity in the soil very rapidly, by comparison with sodium chlorate. While decomposing it has a depressing effect on numbers of soil micro-organisms, nitrification, and carbon dioxide production.

A series of growing wheat plots was sprayed with sodium dichromate on July 1, 1932. The wheat yields of that season, and the wheat, oats, barley, rye and flax yields of the following season are shown in Table V. It will be observed that the direct effect of spraying the wheat crop was pronounced, as even the lightest application reduced the grain yield to about one-half of that of the untreated plots. However, it will be observed that no injurious residual effect was carried over into the following season. The effect seemed rather beneficial as the yields, in 1933, were in nearly all cases higher following the heaviest application of sodium dichromate.

TABLE V

EDMONTON FIELD PLOT CROP YIELDS SHOWING DIRECT AND RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF SODIUM DICHROMATE

$Na_2Cr_2O_7$ applied July 1, 1932		Wheat yield 1932	Yields 1933				
			Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	2494	2079	1972	2132	2105	1897
	Grain per acre, bu.	29.5	21.3	54.1	25.5	25.7	11.8
A/2 Conc.	Straw per acre, lb.	1521	2612	3385	2772	2905	2292
	Grain per acre, bu.	14.2	25.8	65.1	37.7	26.2	10.5
A Conc.	Straw per acre, lb.	1379	2585	3171	3544	2852	2574
	Grain per acre, bu.	8.9	26.2	76.8	37.7	31.4	11.1
B Conc.	Straw per acre, lb.	791	3624	3171	3843	3065	2734
	Grain per acre, bu.	4.1	30.2	87.8	50.5	33.8	14.9

Sodium dichromate was applied to a series of fallow plots also, on July 1, 1932. This series included a heavier rate of application than the other series, as shown in Table VI. It seems clear that no injurious effect was carried

TABLE VI

EDMONTON FIELD PLOT CROP YIELDS SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF SODIUM DICHROMATE

$Na_2Cr_2O_7$ applied July 1, 1932		Yields 1933				
		Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	3892	3017	3705	4652	3545
	Grain per acre, bu.	30.2	57.5	39.4	44.3	2.6
A. Conc.	Straw per acre, lb.	4612	4433	5305	5238	4366
	Grain per acre, bu.	35.1	120.9	48.9	52.4	5.3
B. Conc.	Straw per acre, lb.	4012	4278	5132	5345	4465
	Grain per acre, bu.	34.6	116.4	46.9	52.6	4.9
C Conc.	Straw per acre, lb.	4065	4238	5118	4718	4630
	Grain per acre, bu.	34.4	111.3	46.6	43.3	3.0

over into the 1933 season, as the grain yields were rather higher on the heaviest or C concentration plots than on the check plots. The yield of the untreated oat plots is apparently irregular, as it is much lower than that of the treated oat plots, and the flax plot yields are somewhat irregular also.

AMMONIUM THIOCYANATE

Field Experiments

Field plot experiments like those carried out with sodium dichromate were also conducted with ammonium thiocyanate. A series of growing wheat plots was sprayed with ammonium thiocyanate early in July, 1932. The wheat yields of that season and the wheat, oats, barley, rye and flax yields of the following season are shown in Table VII.

TABLE VII

EDMONTON FIELD PLOT CROP YIELDS SHOWING DIRECT AND RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF AMMONIUM THIOCYANATE

NH_4CNS applied July, 1932		Wheat yield 1932	Yields 1933				
			Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	3181	1546	2665	2825	2212	2095
	Grain per acre, bu.	27.4	16.0	64.3	33.3	23.3	8.8
A/2 Conc.	Straw per acre, lb.	738	2825	4424	4488	4904	4717
	Grain per acre, bu.	0.9	26.6	109.7	52.5	52.4	9.0
A Conc.	Straw per acre, lb.	781	3358	3891	4237	6108	4728
	Grain per acre, bu.	1.5	30.2	109.7	48.9	16.6	8.9
B Conc.	Straw per acre, lb.	805	1492	3465	3747	5863	4793
	Grain per acre, bu.	1.2	11.5	86.2	31.3	3.8	3.8

The direct effect of spraying the wheat crop was greater than in the case of sodium dichromate, as even the lightest application greatly reduced the straw yield, and reduced the wheat grain yield to a small fraction of that of the untreated plots.

The total crop yield in 1933 was increased in nearly all cases by the previous year's applications of ammonium thiocyanate, and this may be attributed, in part at least, to the nitrogen in this chemical. The beneficial effect upon total yield was generally greater following the lighter than following the heavier applications, and, in the cases of wheat, rye, and flax the grain yield was reduced by the heavier application. This indicated that complete decomposition of the thiocyanate had not taken place in the soil to which the heavier application had been made, and analyses of these plots reported in Table IX show that the thiocyanate did not decompose completely during

TABLE VIII

EDMONTON FIELD PLOT CROP YIELDS SHOWING RESIDUAL EFFECTS OF DIFFERENT APPLICATIONS OF AMMONIUM THIOCYANATE

NH_4CNS applied July, 1932		Yields 1933				
		Wheat	Oats	Barley	Rye	Flax
Check	Straw per acre, lb.	5889	4665	4492	6225	4044
	Grain per acre, bu.	38.6	108.7	42.2	52.1	7.5
A Conc.	Straw per acre, lb.	6964	5278	5038	7558	4785
	Grain per acre, bu.	42.1	95.6	33.9	53.6	1.7
B Conc.	Straw per acre, lb.	5674	5878	4438	8171	
	Grain per acre, bu.	28.0	57.2	29.7	15.9	
C Conc.	Straw per acre, lb.	4560	5085	5326	6377	
	Grain per acre, bu.	13.0	41.5	10.1	10.4	

TABLE IX

UNDECOMPOSED THIOCYANATE REMAINING IN SOIL OF EDMONTON FIELD PLOTS TEN MONTHS AFTER APPLICATION

NH_4CNS treatment July, 1932		Sampling depth, ft., May 22, 1933	NH_4CNS recovered May 22, 1933	
			% of amount applied	lb. per acre
Check		0–1	00.0	00.0
		1–2	00.0	00.0
		2–3	00.0	00.0
A/2 Conc.	163 lb./acre	0–1	12.5	20.3
		1–2	00.0	00.0
		2–3	00.0	00.0
A Conc.	327 lb./acre	0–1	18.7	61.1
		1–2	00.0	00.0
		2–3	00.0	00.0
B Conc.	654 lb./acre	0–1	12.5	81.7
		1–2	3.1	20.3
		2–3	00.0	00.0

the season of application. There was some thiocyanate left in May, 1933, approximately ten months after the thiocyanate had been applied, especially in the soil to which the heavier applications had been made.

The color of the crops also showed that thiocyanate was still present in the soil in the early part of the 1933 growing season, as it was observed that the treated plot crops were yellowish, and that there were some withered leaf tips, especially in the plots to which the heaviest application had been made.

The heaviest application (B concentration) produced an increased growth of lamb's quarters, *Chenopodium album*, L., and ball mustard, *Neslia paniculata*, L., in 1933, especially in the flax plots. The growth of these annual weeds was apparently stimulated by the nitrogen in the ammonium thiocyanate.

Nitrates were determined in these plots in May, 1933, and the quantities of nitrate present in the soil to a depth of three feet are shown in Table X. These analyses indicate that nitrification was not prevented by the thiocyanate in the cases of the two lighter applications, but that nitrification was retarded in the case of the heaviest application or B concentration where the nitrogen added as ammonium thiocyanate would otherwise have been expected to produce the greatest accumulation of nitrates.

TABLE X

NITRATE NITROGEN IN SOIL OF EDMONTON FIELD PLOTS TREATED, TEN MONTHS PREVIOUSLY, WITH AMMONIUM THIOCYANATE

NH_4CNS treatment June 1932		Sampling depth, ft., May 22, 1933	Nitrate nitrogen p.p.m., May 22, 1933
Check		0–1	15.1
		1–2	4.2
		2–3	2.7
A/2. Conc.	163 lb./acre	0–1	25.0
		1–2	7.5
		2–3	2.9
A Conc.	327 lb./acre	0–1	24.8
		1–2	6.6
		2–3	5.5
B Conc.	654 lb./acre	0–1	15.1
		1–2	8.5
		2–3	7.3

Ammonium thiocyanate was also applied to a series of fallow plots in early July, 1932. This series included a heavier rate of application than the other series, as shown in Table VIII. The B concentration reduced the grain yield considerably in 1933, and the C or highest concentration reduced the grain yield very much. The straw yield was not generally reduced by the heaviest application, and was in all cases increased by the lightest application. These increases may be attributed to the nitrogen in this chemical. The decrease in grain yield of the B, and especially of the C concentration plots indicated that the thiocyanate had not decomposed completely in the plots to which the heavier applications had been made. This was indicated, also, by the fact that in the early part of the 1933 growing season the treated plot plants were yellowish in color, and, in certain cases, the leaves were withered. This effect seemed to disappear in the latter part of June and the beneficial effect of the nitrogen in the ammonium thiocyanate became apparent. The treated plot crops then became greener than the untreated, and the annual weeds present developed rapidly. The B and C concentration plots under wheat, oats, barley and rye were heavily infested with ball mustard, *Neslia paniculata*, L., and lamb's quarters, *Chenopodium album*, L., in 1933, and, under flax, even the A concentration plots were heavily infested with these weeds. The weeds, no doubt, had much to do with the reduced yields. The flax in the B and C concentration plots was almost completely choked out by these weeds. It seemed as though the growth of annual weeds was considerably stimulated by this nitrogenous weed killer, and that its concentration was not sufficient to destroy the weed seeds.

This stimulation of weed growth was again observed in 1934. Wheat was sown on the plots in May. In June and July it was noted that the wheat plant leaves on the treated plots were taller and darker green than the leaves in the untreated plots, especially in the case of the C concentration plots, to which the heaviest applications of ammonium thiocyanate had been made. This shows that the toxic effect of the thiocyanate had disappeared, and that the growth was stimulated by the nitrogen in this chemical. However, the growth of lamb's quarters, *Chenopodium album*, L., was also much greater on the treated plots, and this was especially true of the C concentration plots. Crop yields were not taken in 1934 as an outbreak of loose smut of wheat made it necessary to destroy the crop about the middle of July.

TABLE XI

AMMONIUM THIOCYANATE RECOVERED FROM SOILS BY LEACHING TEN DAYS AFTER IT HAD BEEN APPLIED (1000 GM. SOIL IN EACH CONTAINER)

NH_4CNS applied		NH_4CNS recovered	
		Gm.	% of that applied
Winterburn sandy loam			
Check		0.00	
A/2 Conc.	.13 gm.	0.049	37.7
A Conc.	.26 gm.	0.127	48.8
B Conc.	.52 gm.	0.272	52.4
Edmonton loam			
Check		0.00	
A/2 Conc.	.13 gm.	0.055	42.1
A Conc.	.26 gm.	0.122	47.1
B Conc.	.52 gm.	0.248	47.7
Breton wooded soil			
Check		0.00	
A/2 Conc.	.13 gm.	0.020	15.6
A Conc.	.26 gm.	0.069	26.5
B Conc.	.52 gm.	0.193	37.2

AMMONIUM THIOCYANATE

Laboratory Experiments

A laboratory experiment to compare the rates at which ammonium thiocyanate decomposes in different soils was conducted with Winterburn sandy loam, Edmonton black loam, and Breton gray wooded loam, as shown in Table XI. The treated soils were kept at optimum moisture and room temperature for ten days. They were then leached with water and the quantities of ammonium thiocyanate present in the leachate were determined. It was observed that the thiocyanate decomposed rapidly under those favorable conditions in all three soils.

Another somewhat similar laboratory experiment was conducted with the same three soils. In this case, however, tumblers of treated soil, incubated at optimum moisture and room temperature, were removed for extraction and analysis every two weeks. Analyses of the water extractions are given in Table XII, and it will be observed that after six weeks incubation under favorable conditions, the thiocyanate had apparently decomposed completely in all except the sample of gray wooded soil to which the heaviest application of thiocyanate had been made. This experiment indicates that thiocyanate decomposes more rapidly in a fertile soil rich in organic matter than in poorer soils, as it disappeared most rapidly from the Edmonton black loam.

TABLE XII

AMMONIUM THIOCYANATE RECOVERED IN WATER EXTRACTIONS OF TREATED SOILS AFTER DIFFERENT PERIODS OF INCUBATION (TUMBLER EXPERIMENT)

Gm. NH_4CNS applied, per 100 gm. of soil	2 Weeks		4 Weeks		6 Weeks		8 Weeks	
	Gm.	% of that applied	Gm.	% of that applied	Gm.	% of that applied	Gm.	% of that applied
		Edmonton black loam						
Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A/2 Conc. .013	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A Conc. .026	0.0032	12.5	0.0	0.0	0.0	0.0	0.0	0.0
B Conc. .052	0.0049	9.4	0.0	0.0	0.0	0.0	0.0	0.0
		Winterburn sandy loam						
Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A/2 Conc. .013	0.0032	25.0	0.0	0.0	0.0	0.0	0.0	0.0
A Conc. .026	0.0065	25.0	0.0049	18.7	0.0	0.0	0.0	0.0
B Conc. .052	0.0106	20.4	0.0081	15.6	0.0	0.0	0.0	0.0
		Breton gray wooded loam						
Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A/2 Conc. .013	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A Conc. .026	0.0065	25.0	0.0	0.0	0.0	0.0	0.0	0.0
B Conc. .052	0.0162	31.2	0.0081	15.6	0.0032	6.2	0.0	0.0

NOTE: *No NH_4CNS was recovered in the tenth and twelfth week incubations. The soil cultures were kept at optimum moisture and 28° C.*

Determinations were made of nitrate as well as thiocyanate in these soil extracts, and the nitrate figures are shown in Table XIII. The quantities of nitrate were reduced by the thiocyanate in all three soils, especially at first. The heaviest application reduced nitrification for a period of eight weeks in all three soils, and, in the Breton gray loam, even the lightest application reduced the nitrification for at least twelve weeks. This was probably due to the toxicity of the thiocyanate, as the nitrogen in this chemical would otherwise be expected to produce a greater accumulation of nitrate.

TABLE XIII

EFFECT OF AMMONIUM THIOCYANATE ON SOIL NITRIFICATION (TUMBLER EXPERIMENT)

Treatment, gm. NH_4CNS per 100 gm. soil	Period of incubation					
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
	Winterburn sandy loam: p.p.m. nitrate nitrogen					
Check	33	40	51	54	54	67
0.013	28	38	52	43	54	77
0.026	11	37	57	40	61	80
0.052	7	14	32	41	78	100
	Edmonton black loam: p.p.m. nitrate nitrogen					
Check	52	57	63	71	91	100
0.013	29	37	45	71	105	105
0.026	24	37	44	71	105	133
0.052	23	25	17	61	91	100
	Breton gray loam: p.p.m. nitrate nitrogen					
Check	6	20	28	20	62	81
0.013	5	4	9	10	15	43
0.026	trace	3	9	5	8	28
0.052	trace	trace	6	4	5	9

Discussion

In the following discussion of the effects on soils and subsequent crop growth of chemicals used for weed control, some of the experiments reported in Part I of these studies (3) are referred to as well as the more recent observations.

The experiments show that sulphuric acid and copper sulphate have no injurious residual effect upon the soils studied. It is altogether likely that much larger quantities than those required to destroy annual weeds could be applied to these soils without causing injurious after-effects.

Sulphuric acid can be obtained at a lower cost than copper sulphate and another advantage of the acid is that the copper sulphate is relatively ineffective when rain falls within a few hours after spraying, whereas the sulphuric acid, by its quicker action, destroys the weeds effectively under the same conditions (2).

It is interesting to observe that although the sodium chlorate applied in 1931 did not injure the following year's crop of wheat nearly as much as the chlorate applied a year earlier, the toxic effect of the 1931 application lasted rather longer than that of the 1930 application. The reduction in yield of the second or 1933 crop of wheat following the 1931 application was greater than in the case of the corresponding crop following the 1930 application. The high rainfall of the late summer of 1931, which greatly reduced the injury of the 1931 application to the following year's crop, probably removed most of the chlorate remaining in the plots treated with sodium chlorate in 1930.

The presence of toxic quantities of chlorate in treated soils may be determined not only by its effects on crops in the field, but also by chemical analysis of the soils, and by its effect upon the growth of seedlings in small pots of the soil (3). The Winogradsky plaque culture method may be used also as a rapid microbiological test for the effect of chlorate in soils (4).

It has been pointed out by various workers that sodium chlorate is too expensive for use on large areas (7). The comparative cost and effectiveness of tillage and chlorates for the eradication of morning glory, Canada thistle and perennial sow thistle have been discussed recently by Tingey (9).

Sodium dichromate is highly toxic when first applied, or when placed in direct contact with the growing plant. It was much more toxic to wheat than an equal concentration of sodium chlorate in water cultures, and an application of about 160 lb. per acre (the lightest application) injured the growing wheat crop considerably when applied about the first of July. Sodium dichromate decomposes in the soil too rapidly to be of great value as a perennial weed killer, as it loses its toxicity, apparently, before it has reached and destroyed the underground root systems of many perennials. It might have value as a perennial weed killer when mixed with the less readily decomposable chlorate.

Ammonium thiocyanate applied to growing wheat reduced the yields even more than the corresponding applications of sodium dichromate, and, in contrast to the dichromate, the thiocyanate did not decompose and lose its toxicity completely during the season of application. However, it does decompose and lose its toxicity much more rapidly than sodium chlorate.

The effects of different applications of sodium chlorate and ammonium thiocyanate upon subsequent sowings of wheat in soil pot cultures in which the moisture content of the soil was carefully maintained have been reported recently by Bissey and Butler (1). They observed that with an application equivalent to 800 lb. per acre of sodium chlorate the toxicity persisted approximately two years, whereas the toxicity of an 800 lb. application of ammonium thiocyanate disappeared within two months, under these conditions of optimum moisture and no leaching. In spite of its relatively rapid decomposition in the soil it has been observed by Harvey (6) and other workers that ammonium thiocyanate is almost or quite as effective a destroyer of perennial weeds as sodium chlorate.

According to Harvey (6) the injurious after-effect of the chemical can be removed by applying a ferric salt, which reacts with the chemical to form non-toxic colloidal ferric thiocyanate. While this, of course, would add to the cost of the method, it offers a means for removal of the toxic action before it is desired to plant a crop.

Growth of certain common annual weeds was evidently stimulated by the nitrogen in the ammonium thiocyanate applied a year earlier, and its concentration was not sufficient to destroy the weed seeds, as a greater growth of lamb's quarters, *Chenopodium album*, L., and ball mustard, *Neslia paniculata*, L., occurred in the treated than in the untreated plots. The greatest growth of these weeds occurred in the plots which had in the previous season received the heaviest applications of ammonium thiocyanate. The flax crop was almost completely choked out by the weeds in these plots, for, as pointed out by Pavlychenko and Harrington (8), flax cannot compete with weeds as successfully as most of our common cereals.

If perennial weeds are successfully destroyed by a chemical, it may not be a serious objection if some annual weeds start to grow on the treated patches before the toxic effect of the chemical has entirely disappeared. However, the nitrogenous character of ammonium thiocyanate may be a disadvantage rather than an advantage, as subsequent growth of annual weeds on plots treated with sodium chlorate and sodium dichromate was not nearly as great as in the case of plots treated with the thiocyanate. It was observed by Grau (5) that when ammonium thiocyanate was used for the purpose of controlling turf weeds, it tended to encourage certain weeds.

It was pointed out in Part I of these studies (3) that when placed in contact with germinating wheat seeds, and in contact with growing wheat plant roots, in water culture experiments, sodium dichromate is much more toxic to wheat than sodium chlorate in equal concentration. According to Bissey and Butler (1) ammonium thiocyanate is similarly much more toxic than sodium chlorate in water cultures.

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MICROBIOLOGICAL STUDIES OF APPALACHIAN UPLAND PODSOL SOILS

I. EFFECTS OF PHYSICAL AND CHEMICAL TREATMENTS[1]

By P. H. H. Gray[2] and H. J. Atkinson[3]

Abstract

A study has been made of the effects of fertilizers and limestone, of fallowing, and of deep ploughing, upon certain aspects of microbial activity in representative Appalachian podsol soils at three farms in the Eastern Townships region of Quebec Province. The results show that the evolution of carbon dioxide and numbers of bacteria and actinomyces were not altered by any simple fertilizer applied annually for two years. Limestone, at the rate of six tons per acre, increased the numbers of micro-organisms during the two years of the experiment. Deep ploughing reduced both carbon dioxide and bacterial numbers in soils ordinarily ploughed to a normal depth.

Studies have also been made to determine the effects of some other chemical treatments upon the soil microflora. Carbon dioxide, bacterial numbers and nitrification of soil nitrogen were increased in field plots after treatment with calcium oxide, sodium carbonate and sodium hydroxide, alone or in combination; the effects lasted through two seasons.

The nature and distribution of podsol soils in Quebec Province have been discussed in previous papers (2, 4, 5). The high percentage of organic matter in these soils is a potential source of fertility, provided some means can be found of making its valuable food elements more readily available for plants. Since the decomposition of organic matter is largely due to microbial activity, treatments that stimulate the action of micro-organisms should help to increase soil fertility. The authors present in this paper the results of microbiological studies in field plots that have, during four years, received such treatments as might be expected to stimulate microbial activity.

A study was first made of the effects of soil treatments and fertilizers in common use, namely, ground limestone; nitrogenous, phosphatic and potassic fertilizers; deep ploughing in comparison with normal ploughing; and the effects of cultivated summerfallow. In the spring of 1931, sets of plots were laid out on three farms in the Eastern Townships region of Quebec; one in Brome County, one in Compton County, and one in Sherbrooke County. The location of these areas is shown in Fig. 1. In each case the soil was of the typical heavily leached podsol type, and had received no previous treatment except organic manure.

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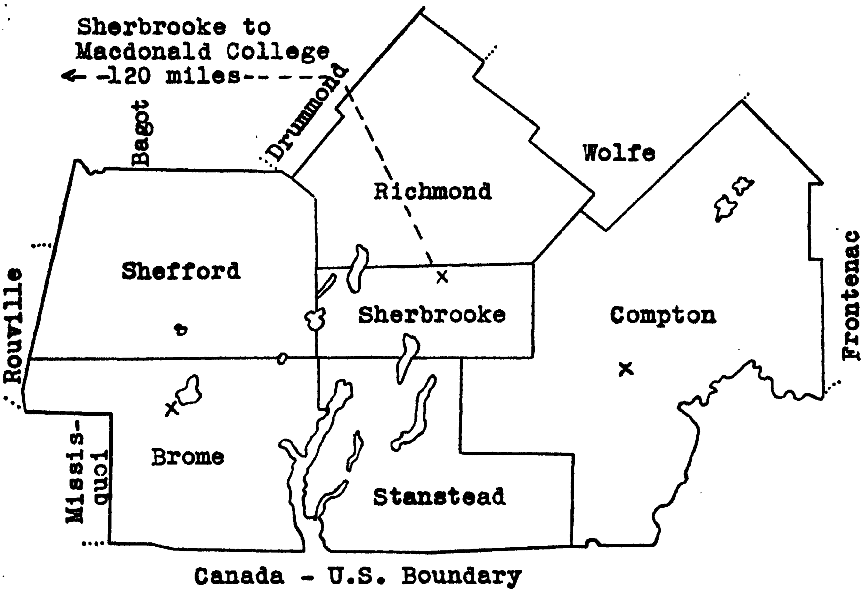

FIG. 1. *Sketch map showing location of plots* (X) *in the Eastern Townships region of Quebec.*

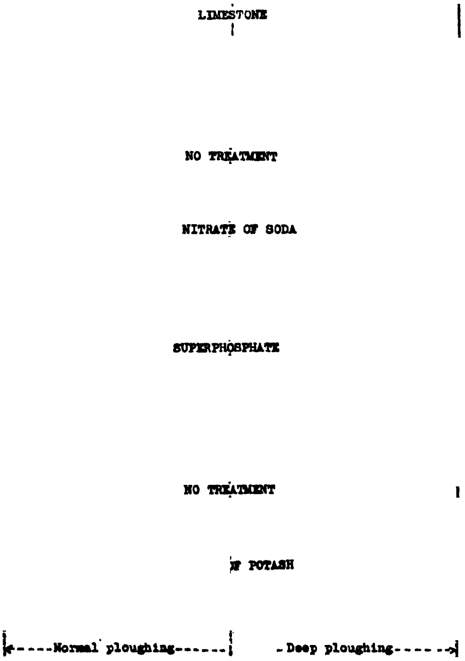

FIG. 2. *Plan of plots in experiments of 1931.*

An area of one acre was subdivided and treated according to the plan shown in Fig. 2. In one direction the area was divided into two equal parts, one-half being ploughed to a depth of 10–12 in., the other half to the customary depth, five or six inches. In the other direction the area was divided into six equal parts, as shown in the plan. Each treatment thus covered one-twelfth of an acre.

Oats were sown on the Brome County and Compton County plots, and corn on the Sherbrooke County plots.

The rates at which the treatments were applied per acre were as follows:-

Pulverized limestone (satisfying the lime requirement test by Jones' method)	6 tons
Nitrate of soda	200 lb.
Superphosphate (16%)	800 lb.
Muriate of potash	200 lb.

The chemicals were harrowed in immediately after application. The fallow plots were cultivated during the summer of 1931.

It was decided to limit the investigation to changes brought about in the soil itself by the treatments used. Representative soil samples for investigation were obtained by boring into the soil to a depth of six inches, by means of a piece of iron piping having an inside diameter of about one and three-quarter inches. When the sampling tool was withdrawn, the core of soil was removed by hammering on the outside of the pipe. Ten such samples, well distributed over the area, were taken from each one-twelfth acre plot and thoroughly mixed on a rubber sheet. A representative sample of about three pounds was taken from this. All samples were brought to the laboratory in small covered tins immediately after being taken and their examination was begun within 24 hr. of the time of sampling. Each set of plots was sampled four times during the growing season, at intervals of six weeks, the first samples being taken in each case before the application of the treatments.

The investigation was continued during 1932. The plots were ploughed in the fall of 1931, in the same way as had been done previously. In the spring of 1932 the applications of nitrate of soda, superphosphate, and muriate of potash were repeated. The plots that were fallowed during 1931 were cropped in 1932, Swede turnips being grown at all three places.

The biological determinations included the numbers of bacteria and actinomyces, the evolution of carbon dioxide, and the amount of nitrate nitrogen in the fresh samples, by the methods described in a previous paper (2).

Early in the work a test was made to determine the variation in biological factors within a single plot. For this purpose ten samples of four cores each were taken from each of two of the one-twelfth acre plots in Brome County; for the necessary comparison, a composite sample of ten single cores was made in the usual way at the same time. The ten samples and the composite sample from each plot were analyzed for carbon dioxide, and the numbers of bacteria were estimated.

The amounts of carbon dioxide and the numbers of micro-organisms found in these samples are shown in Table I.

TABLE I

CARBON DIOXIDE, BACTERIA AND ACTINOMYCES IN SOIL SAMPLES

	Carbon dioxide, mg. per 100 gm. per 100 hr.		Bacteria and actinomyces, millions per gm.	
	Average of 10 subsamples	Composite	Average of 10 subsamples	Composite
Plot 2	17.78 ± .743	17.52	16.59 ± .949	17.48
Plot 6	12.92 ± .627	12.63	13.60 ± 1.1296	15.54

It appeared from this test that the technique of sampling by means of ten single-core samples would be representative of biological activity in a plot.

It should be noted, however, that Plot 3 (no treatment, cropped) was divided into half plots distributed as shown. There was no appreciable difference between the analyses for each factor in these two smaller plots, in the experiment in Brome County. In the results to be considered below, this subdivision, and also that of Plot 2 (no treatment, fallow in 1931), may be a factor that would give different weight in an analysis of variance. A further limitation to accurate analysis must be observed in regard to the distribution of chemical treatments (arrangement of plots) at each place; these were not randomized within each acre area. The similarity of biological activity, however, at the three places, in regard both to levels and to direction of changes during two seasons, studies of which will form the subject matter of a second paper in this series, suggests that the results obtained from the plots receiving no chemical treatments can be accepted as representing reliable values.

Limestone was the only treatment that appeared to affect the biological activity in the plots. The results in regard to bacterial numbers, carbon dioxide, and nitrates in fresh samples, are shown in Table II, in which the plots treated with limestone are compared with the untreated cropped plots. In view of the fact that seasonal changes of numbers between the first and the second samples might have been masked by the treatment, the values shown are the averages of the three samples taken after treatment in the first year, and of the four samples taken in the second year. In regard to carbon dioxide, the results for the second year only are shown, since the chemical action of the limestone, from which carbon dioxide was freed by soil acids, obviously masked any differences due to biological action for at least twelve weeks after

TABLE II

MICROBIOLOGICAL RESULTS IN THE EXPERIMENTS OF 1931

Locality	Brome		Compton		Sherbrooke	
Ploughing	Normal	Deep	Normal	Deep	Normal	Deep
		Results in 1931				
Numbers of bacteria and actinomyces; millions per gm.						
Limestone	14.15	18.30	20.11	20.12	26.75	22.07
No treatment	13.98	11.09	10.42	12.94	14.71	10.72
		Results in 1932				
Numbers of bacteria and actinomyces; millions per gm.						
Limestone	13.54	15.62	15.03	12.66	37.68	19.89
No treatment	13.29	10.51	8.58	10.25	18.94	14.60
Carbon dioxide, mg. per 100 gm. per 100 hr.						
Limestone	32.25	39.55	49.03	36.60	80.05	53.90
No treatment	27.05	15.43	22.03	21.35	50.68	32.70
Nitrate nitrogen, parts per million						
Limestone	12.72	9.81	43.09	30.91	29.36	31.91
No treatment	10.63	8.21	30.25	26.92	24.60	32.99

treatment. Nitrate nitrogen was used up rapidly during the first year, and no values were therefore available in the later samples, except in those from the fallow plots; the values shown in Table II are the averages of the four samples taken in 1932, in all of which nitrates were found to be present.

It is clear from the results shown in Table II that limestone increased activity in these acid soils, and that the effects lasted through a second season.

In view of the fact that bacterial numbers are known to fluctuate extensively in untreated field soil, it did not seem reasonable to determine the degree of significance of the effects produced by limestone, simply on the basis of deviation of values from the first samples in the plots under consideration. An analysis of variance (1) was therefore made, using all of the values obtained from all of the plots. Bearing in mind the limitations mentioned above in respect to the arrangement of the plots, and also the evident similarity of the results obtained at the three places, the analysis has been used to determine also the significance of the effects of the two depths of ploughing. The analysis takes into consideration the distribution of the areas in widely separated localities, the two seasons, and the four periods of sampling at intervals of six weeks during two seasons, and also interactions between any two such factors as appeared to be important sources of difference, namely, treatments, ploughing, and places.

The analysis in Table III shows on a basis of probability, (P = .05), that the limestone treatment alone was effective in increasing bacterial numbers. There appears also to be a significant reduction in numbers through ploughing to a greater depth than is normally the practice. Similar results were given by the analysis of the values for carbon dioxide; deep ploughing significantly depressed biological activity, and no differences were found between treatments (exclusive of limestone).

TABLE III

ANALYSIS OF VARIANCE FOR MICRO-ORGANISMS AND FOR CARBON DIOXIDE
(Experiments of 1931)

	Micro-organisms		Carbon dioxide	
	Degrees of freedom	Sums of squares	Degrees of freedom	Sums of squares
Treatments	5	1601.16	4	1104.75
Ploughing	1	148.06	1	1138.40
Places	2	1636.13	2	5579.70
Years	1	56.39	1	359.91
Sampling	3	252.89	3	1008.28
Interactions:—				
Treatments × ploughing	5	119.88	4	239.40
Treatments × places	10	504.35	8	502.07
Ploughing × places	2	334.19	2	966.75
Residual	258	6330.62	214	20006.86
Total	287	10983.67	239	30906.12
Mean	14.021 ± 1.152		28.85 ± .624	
Standard deviation	4.953 ± .206		9.669 ± .441	

TABLE III—*Concluded*

MEANS

Treatments			Ploughing		
	Micro-organisms	Carbon dioxide		Micro-organisms	Carbon dioxide
Limestone	18.894		Normal	14.738	31.03
Fallow	11.548	24.86	Deep	13.304	26.67
Check	12.509	29.42			
Nitrogen	14.422	30.96			
Phosphorus	13.048	30.35			
Potash	13.705	28.65			
Difference required for significance	3.433	6.116		1.114	2.446

In searching for a cause for reduction of microbial activity brought about by deep ploughing, two possible factors have to be considered. The reduction found in the first samples must be due, probably solely, to dilution of the upper layer of soil with a proportionate amount of soil from the lower layer, in which the number of micro-organisms is much less than in the well tilled upper layer, a fact long known to all students of soil microbiology. It may not however be quite clear why this mechanical reduction should persist throughout two seasons, especially in view of the fact that the deeply ploughed half-acres of land were again turned over to the same depth in the second year, thus, presumably, effecting a more thorough mixing of the organic matter from which the micro-organisms obtained their material for energy and growth. With the data at present available it seems feasible to postulate that the reduced level of activity in the deeply ploughed plots in the second year can definitely be ascribed to the cultivation and not to changes induced by season. It may be stated that with both deep and shallow ploughing, biological activity in the second year was considerably less in the Brome and Compton soils, but considerably higher in the Sherbrooke soil; differences in bacterial numbers were in the same direction in all cases, but of a smaller order of difference; in spite of these irregular changes from one year to the next, deep ploughing, as has been shown, was a significant factor.

In addition to mechanical dilution, there is the factor of nutrition. In this case, the only available data regarding the nutrition of the organisms is contained in chemical analyses made from the first samples taken before the treatments were applied. In this connection, it seemed useful to determine whether or not the constituents analyzed were similarly reduced by deep ploughing. It was found that both carbon and nitrogen were thus mechanically diluted to a significant degree. In regard to mineral nutrients, there was a definite reduction in the amount of calcium, but magnesium and phosphorus were altered irregularly; the total of these differences was probably negligible. Whatever effects deep ploughing had on the alteration in the proportion of nutrients, it cannot be claimed that the differences found had any biological significance; such effects, if any, would only be felt after a considerably longer time than two seasons.

It will be noted, in Table II, that numbers of micro-organisms in the deeply ploughed untreated plot in Compton County were higher than those in the normally ploughed plot, in both years of the experiment. A difference of a similar order was obtained after summarizing the results from all of the plots at this farm. The results were similar in the case of the analyses for carbon dioxide, excluding the plots treated with limestone. The actual mean values for these two biological factors are shown in Table IV.

TABLE IV

Ploughing	Micro-organisms, millions per gm.			Carbon dioxide, mg. per 100 gm. per 100 hr.		
	Brome	Compton	Sherbrooke	Brome	Compton	Sherbrooke
Normal	13.59	10.95	19.42	27.06	26.86	39.17
Deep	12.73	12.04	15.14	20.41	28.15	31.46

It appears to be worth recording that it had been the practice at the Compton County farm for many years to plough to a greater depth than was customary elsewhere. After a considerable number of seasons, therefore, deep ploughing of these soils may prove beneficial to microbial activities.

During the winter of 1931-32, chemical investigations in the Macdonald College laboratories (6) had shown that certain strong alkali treatments were effective in increasing the amount of soluble organic matter. It was thought that the use of these treatments in field plots should be studied and their effect on the activity of micro-organisms determined. For this purpose small plots were laid out in the spring of 1932 at each of the three farms, and close to the 1931 plots. Each plot was circular in shape, and enclosed an area of one hundred square feet (radius = 5.64 feet). Twelve such plots were installed at each of the three farms, randomized in two rows of six plots each, and the centres of the plots were exactly thirteen feet apart. The treatments used are shown in Table V. These treatments were applied to the surface of the soil in the spring of 1932, and harrowed in. They were not repeated in the succeeding year. Oats, seeded down with a timothy and clover mixture, was the crop grown at the three places.

Samples of four cores each were taken from each circular plot at intervals of six weeks during the season, at the same time as samples were taken from the plots laid down in the previous year, and the same biological analyses were made in the laboratory. In view of the fact that the areas in Brome County and Sherbrooke County had been previously treated with coarsely ground limestone, unevenly spread, it was decided after one season to limit attention to the plots in Compton County, which had not previously received such treatment. For this reason the results shown in Table V refer only to the plots at the latter place.

TABLE V

TREATMENTS, AND RESULTS OBTAINED, IN THE COMPTON COUNTY EXPERIMENT OF 1932

Plot No.	Treatment	Rate per acre	1932			1933	
			Carbon dioxide[1]	Micro-organisms[1]	Nitrate nitrogen[2]	Carbon dioxide[3]	Micro-organisms[3]
1	Na_2CO_3	1000 lb.	105	267	139	104	147
2	CaO	Lime requirement	236	350	298	156	156
3	Na_2CO_3 CaO	1000 lb. Lime requirement	184	423	565	139	210
4	NaOH	400 lb.	153	336	273	111	151
5	Nil	Nil	100	100	100	100	100
6	NaOH CaO	400 lb. Lime requirement	207	274	361	169	157
7	CaO	Double lime requirement	202	422	285	167	186
8	Na_2CO_3	500 lb.	121	297	258	110	131
9	NaOH	200 lb.	131	183	trace	113	102
10	Na_2CO_3 CaO	500 lb. Lime requirement	201	305	344	146	147
11	NaOH	200 lb. Lime requirement	174	297	170	120	160
12	Na_2CO_3	2000 lb.	153	1355	721	77	396
	Value required for significance		109	120	118		

[1] *Averages of three samplings;* [2] *In fresh samples six weeks after treatment;* [3] *Averages of four samplings.*

In order to obtain a basis for an estimate of the significance of any changes due to treatment within the area enclosing the twelve plots, samples of four cores each were taken from outside the circular area of each of six plots, chosen at random, before the treatments were applied. It was found that a difference of over 9% in the amount of carbon dioxide would be required for significance. In the case of bacterial numbers, it unfortunately proved impossible to determine the limit for significance with the six samples collected at this time, on account of low numbers of colonies and their irregular dispersion on the plates. In view of the comparative variability of carbon dioxide and bacteria in the previous test it seemed justifiable to postulate 20% as the limit. The limit of significance for nitrate nitrogen was found to be 18%.

In reference to the results, as shown in Table V, it appears that every treatment was effective in stimulating biological activity in the first season, with the exception of sodium carbonate at the rate of 1000 lb. per acre, which did not stimulate the production of carbon dioxide. The presence of only a trace of nitrate nitrogen in the sample from plot No. 9 (200 lb. of sodium hydroxide per acre), can only be ascribed to some unknown accidental cause or causes.

The great increase of bacteria in plot No. 12, with 2000 lb. of sodium carbonate, was accompanied by the destruction of all higher plants in this plot.

During the second season the results showed that the treatments were still effective though in all cases to a lower degree in comparison with the untreated plot. In one case only, that of carbon dioxide in plot No. 12, activity was definitely reduced to a value significantly below that of the untreated plot No. 5. Nitrate nitrogen was not determined in the fresh samples during 1933.

The results can be more easily compared by combining the values obtained in the two seasons and expressing them in the form of graphs, as shown in Figs. 3–5. The results have been arranged in order of magnitude, after halving the sums of the proportional values for carbon dioxide and numbers of micro-organisms. The values for nitrate nitrogen remained as quoted in Table V. It will be seen that, with the exception of carbon dioxide in plot No. 12, there is a close agreement as to the comparative effects of the treatments, in the three biological factors studied. It is interesting to note that in the results for the evolution of carbon dioxide the order of effectiveness was calcium oxide, sodium hydroxide, and sodium carbonate, though sodium carbonate with calcium oxide takes a high place.

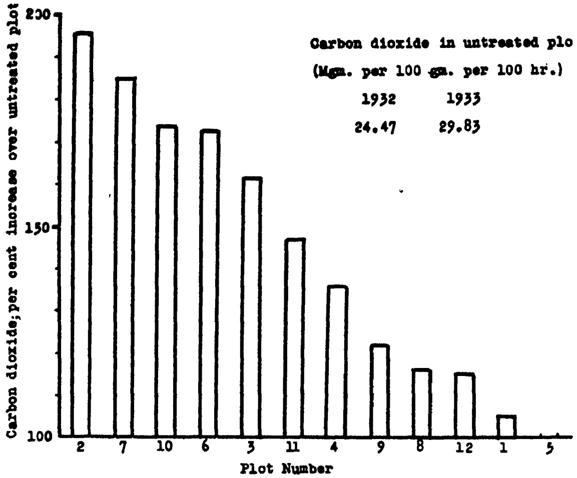

FIG. 3. *Comparative effects of alkali treatments on biological activity in podsol soil. [Mean proportional values for two seasons; untreated plot (No. 5) = 100].*

It is probably not possible to make a close comparison of these results with those of other workers who have reported the effects of similar treatments. The rates of application reported here were calculated on the basis of one acre, but the action of the compounds was probably largely on the soil within the top two or three inches, and may thus have been more concentrated than the figures would imply. For example, with sodium hydroxide, the organic matter on the top of the soil was burned brown. No tests have so far been made to determine the effects at different levels in the soil. It is clear however that they have stimulated the activity of the bacteria commonly associated in the destruction of organic matter, and also the oxidation of the nitrogen compounds in the latter.

In no case was evidence obtained that the calcium oxide induced partial sterilization; at least there was no evidence of any reduction in the biological factors six weeks after its application; such action may have occurred in close contact with the lime, until the excess of oxide had been converted into carbonate. On a basis of lime requirement the calculated amount of calcium oxide, as burned lime applied, was 4000 lb. per acre; this was equivalent to 0.2%, assuming the lime to have been mixed into the soil. In laboratory experiments with caustic lime, Hutchinson (3) showed that 0.1% had no effect on the soil micro-organisms, while 0.5% caused a great increase in bacteria in soils containing from 2 to 3% of calcium carbonate.

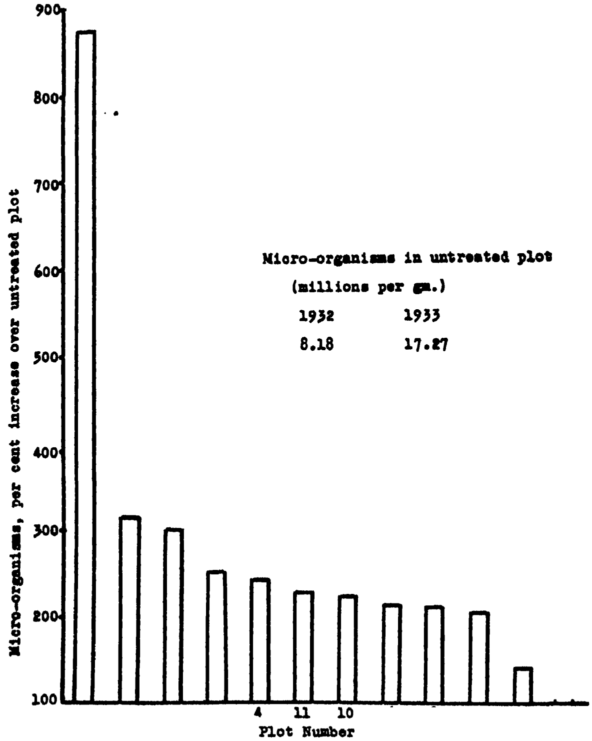

FIG. 4. *Comparative effects of alkali treatments on numbers of micro-organisms in podsol soil. [Mean proportional values for two seasons; untreated plot (No. 5) = 100.]*

The results obtained in the field plots with sodium carbonate and the other soil amendments suggested the value of such chemicals for studies on crop yield in these soils. In the spring of 1933 a duplicated set of plots, each $\frac{1}{100}$ acre in area, was laid out on the Compton County farm. The treatments included, among others, those that were used in the small circular plots laid down in the previous year. In view of the possible importance of sodium carbonate, and also since it is the experience of farmers in the district that unleached wood ashes are beneficial to plant growth, one of the additional treatments comprised a mixture of chemicals, approximating the fertilizing components of high grade wood ashes. The composition was as follows, on a basis of lb. per acre: calcium carbonate 6000, sodium carbonate 600, superphosphate 250, muriate of potash 200. The high amount of sodium carbonate in this mixture, relative to its amount in natural wood ashes, will be remarked.

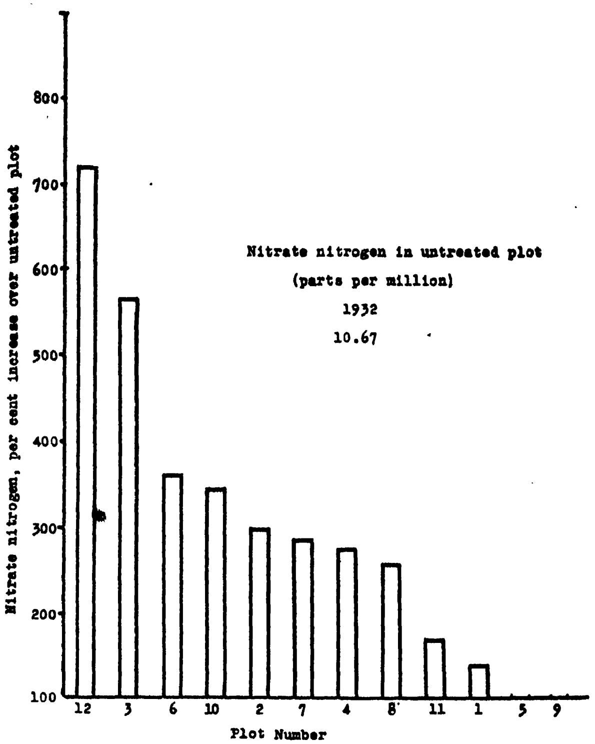

FIG. 5. *Comparative effects of alkali treatments on nitrification in podsol soil. [Proportional values for season of 1932; untreated plot (No. 5) = 100.]*

Samples were taken during 1934, to study the effect of limestone, sodium carbonate, and the "synthetic wood ashes" upon the numbers of micro-organisms one year after treatment. On the dates shown in Table VI, samples of five cores were taken from two similarly treated plots, one in each half-acre, of each of the three treatments selected for study, and from two of the untreated plots. The numbers of micro-organisms were determined by

TABLE VI

NUMBERS OF MICRO-ORGANISMS IN PLOTS OF 1933

Treatment and rate, lb. per acre	Sodium carbonate, 1000	Limestone, 6000	"Wood ashes", 7050	No treatment
Method of counting and date	Micro-organisms, per gm. dry soil			
Plate May 12, 1934	110,160,000	45,055,000	41,595,000	27,590,000
Direct May 12, 1934	2,824,000,000	2,323,000,000	2,024,000,000	1,740,000,000
Plate June 18, 1934	46,400,000	33,805,000	31,900,000	18,605,000

the usual plating method, and also by means of the ratio method in soil films (7). In the table each value represents the mean of the two plots in each case.

It is necessary to point out that the original counts of June 18, in respect of the fresh samples from the sodium carbonate and "synthetic wood ashes" plots, were not reliable; the samples were replated later, and the values shown are those calculated by ratio to a similarly replated sample from the control plots; the true proportionate values are therefore unknown. It is interesting to note that the total cell counts, while not directly proportional to the plate counts, show that the differences between treatments fall into the same order.

The results with limestone and sodium carbonate confirm those found previously, in that the effects of these treatments have lasted for two seasons; the sodium carbonate also, appears to be much superior to limestone; the results with the latter treatment also appear to be comparable with those of the same treatment in the plots of 1931, one year after its application.

Acknowledgment

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INFLUENCE OF WEATHER CONDITIONS ON THE NITROGEN CONTENT OF WHEAT—II[1]

By J. W. Hopkins[2]

Abstract

Supplementing a previous statistical study, coefficients designed to weight observed temperatures in proportion to their assumed effect on respiration were computed from the daily observations for three 3-week periods extending from July 1 to September 1. After allowing for the effect of May and June rainfall there was a moderate but significant partial correlation ($r = +0.33$) between nitrogen content and the sum of the temperature coefficients for the last two periods.

There was a positive correlation ($r = +0.74$) between height of crop and yield of grain, and a negative correlation ($r = -0.50$) between height and nitrogen content. The partial correlation between nitrogen content and yield, after eliminating variations in both associated with height, was negligible ($r = -0.07$), suggesting that reductions in yield due to restriction of the later stages of translocation did not result in significant modification of the nitrogen content of the grain.

Results of the investigation as a whole are briefly discussed.

Introduction

It was at one time thought that the greater part of the nitrogenous constituents of cereal grains was laid down during the earlier stages of kernel formation, subsequent "filling" resulting largely from the translocation of carbohydrates. Retardation or inhibition of this latter process by, for example, drought or hot weather would thus obviously affect the composition of the matured grain.

Chemical studies by Brenchley and Hall (2), Thatcher (8, 9), Woodman and Engledow (11) and more recently by McCalla and Newton (5) have not, however, supported this view. The last-mentioned authors also point out that analytical methods employed by some of the earlier workers may have led to fictitiously high estimates of nitrogen content during the initial stages of kernel formation, and it now appears that although the pericarp tissue may be somewhat richer in nitrogen than the subsequently developed endosperm, the carbon/nitrogen ratio of the translocated material is much less variable than was previously supposed. It might be expected therefore that weather conditions would operate to modify the composition of the crop largely through their effect on the vegetative production of carbohydrates and protein and on the rate of respiration in the developing grain, rather than on the progress of translocation.

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The respiratory effect has been studied by McGinnis and Taylor (6). These writers conclude that although differences in the amount of respiration were definitely incapable of accounting for the whole of the observed variation, they might modify appreciably the percentage nitrogen content of wheat, oats or barley.

Agricultural meteorological studies by the author (3), employing the statistical method, indicated that whereas the yield of wheat grown annually at points in Saskatchewan and Alberta was significantly affected by the amounts of rain falling in May, June and July, the only significant influence of rainfall on nitrogen content was exerted during May and June, a finding which is consistent with the chemical results previously mentioned (2, 5, 8, 9, 11). They did not, however, establish any relation between nitrogen content and the temperature conditions prevailing in July and August, although hot weather at this time might certainly be expected to accelerate respiration.

It was felt that the monthly averages of daily maximum and minimum readings employed perhaps failed to provide a suitable measure of the temperature conditions experienced by the crop, with the result that real effects escaped detection. The outcome of a further analysis, designed to weight the observed temperatures in proportion to their assumed physiological effect (1), is now described. Opportunity is also taken to present the results of a few additional calculations relating growth, yield and nitrogen content.

Physiological Temperature Summations

According to Lundegårdh (4), the respiration of such plant parts (seedlings and foliage leaves) as have been studied at a series of temperatures increases in an approximately exponential manner with temperature up to the lethal point, the results indicating temperature quotients of from 2 to 3. It seemed desirable therefore to ascertain whether there was any correlation between nitrogen content and an exponential function of the temperature, designed to weight the observed values in accordance with this fact.

The actual temperature quotient of the respiration of developing wheat kernels being unknown, the arbitrary value 2.0 was employed. This may have been somewhat conservative. Adopting this value, the assumed respiration at any temperature $t°$ F. will be in the proportion of $2^{\frac{t-32}{18}}$ to that at 0° C. Substitution of the maximum and minimum temperatures recorded on any day in this expression yielded maximum and minimum temperature coefficients, which were summed to give a daily temperature coefficient. These daily sums were then averaged over three 3-week periods, namely July 1–21, July 22–August 11, and August 12–September 1. The average values thus obtained at each station in each season for which crop data were available are shown in Table I, and provided three variables whose relation to nitrogen content was to be investigated.

TABLE I

Temperature coefficients

Station	Year	July 1–21	July 22-Aug. 11	Aug. 12-Sept. 1	Station	Year	July 1–21	July 22-Aug. 11	Aug. 12-Sept. 1
Lacombe, Alberta	1915	5.48	7.97	7.59	Indian Head, Saskatchewan	1928	7.76	8.52	6.11
	17	8.10	6.36	6.98		29	8.83	10.14	9.58
	21	7.32	7.49	6.83		31	8.23	9.87	9.10
	22	7.65	7.91	7.14		32	8.22	9.72	7.66
	25	8.62	8.12	6.22					
	28	6.96	8.12	5.26	Scott, Saskatchewan	1915	6.23	8.37	8.38
	30	8.28	8.01	7.29		17	9.28	7.72	7.35
	31	6.71	7.24	7.12		18	8.57	8.04	6.94
	32	6.18	8.12	7.11		21	8.36	7.37	7.72
						23	7.88	5.09	6.94
Lethbridge, Alberta	1915	6.23	8.29	8.77		24	8.80	6.40	6.34
	16	7.89	6.98	7.02		25	7.91	8.25	6.69
	17	9.44	7.87	7.95		26	7.71	7.28	6.75
	21	8.26	8.04	7.92		29	7.61	8.70	8.54
	22	7.58	8.48	7.92		30	7.57	7.85	7.67
	25	8.83	7.91	6.74		31	6.98	7.64	7.99
	26	9.05	8.02	6.93		32	6.38	8.80	7.33
	27	6.82	6.62	7.72					
	28	6.60	8.83	5.96	Rosthern, Saskatchewan	1915	6.14	8.20	7.82
	29	8.13	9.41	9.49		21	7.90	7.32	7.87
	30	8.58	9.21	8.13		22	7.71	9.26	7.49
	31	7.58	8.98	9.13		24	8.95	6.75	6.49
	32	7.60	9.61	7.29		25	8.20	8.87	6.96
						26	8.51	7.86	7.05
Indian Head, Saskatchewan	1915	6.31	8.65	7.90		27	7.32	7.42	7.93
	18	8.35	7.30	6.63		28	7.97	8.62	5.70
	24	8.54	6.65	6.45		29	8.34	7.54	8.48
	25	8.74	9.11	6.67		30	8.51	8.28	8.51
	26	9.13	8.09	7.86		32	7.16	8.63	7.47

Relation between Temperature Coefficients and Nitrogen Content

A preliminary study of the relation was made by determining the regression of nitrogen content on the temperature coefficient for each period independently, *i.e.*, neglecting in each case any associated effect of temperature in the two other periods. As a significant correlation between nitrogen content and May and June rainfall had already been demonstrated (3), it was necessary to determine the partial regression of nitrogen content on the temperature coefficient after allowing for the association of both with the rainfall during these months. The nitrogen content of the various harvests and the amounts of rain recorded will be found in Tables I and II of the author's earlier paper on this subject (3), and the method of calculation of the seasonal variance of the observed quantities about the means for the different stations is indicated in Section 3 of that paper.

Calculation of the necessary sums of squares and products gave the following normal equations in b_0 and b_1, the partial regression coefficients of nitrogen content on May and June rainfall and on the temperature coefficient for the period July 1–21, respectively:

$$196.8993b_0 - 47.0706b_1 = -13.3427$$
$$-47.0706b_0 + 40.5768b_1 = 3.0289$$

The resulting value of b_1, -0.005% nitrogen per unit increase in the temperature coefficient, is negligible.

Replacing the temperature coefficients for the first period by those for the second gave

$$\begin{aligned} 196.8993b_0 - 5.5323b_2 &= -13.3427 \\ -5.5323b_0 + 37.0529b_2 &= 3.4191 \end{aligned}$$

resulting in $b_2 = +0.082 \pm 0.038$, the estimated standard error of b_2, s_{b_2}, being calculated from the relation

$$s_{b_2} = \sqrt{\frac{s^2\Delta_{22}}{\Delta}}$$

where s^2 is the mean square residual and Δ_{22} is the minor obtained by eliminating the second row and column of the determinant Δ formed from the numerical coefficients on the left hand side of the preceding equations (10, sec. 122).

Similar computations employing the temperature coefficients for the third period, August 12–September 1, had as their outcome

$$\begin{aligned} 196.8993b_0 - 5.1445b_3 &= -13.3427 \\ -5.1445b_0 + 39.7915b_3 &= 2.7894 \end{aligned}$$

and $b_3 = +0.062 \pm 0.037\%$ nitrogen per unit increase in the temperature coefficient.

There was therefore some indication that above-average temperature coefficients in the last two periods were associated with above-average nitrogen content. Seasonal fluctuations in the temperature coefficients for these two periods proved, however, not to be statistically independent; consequently, a portion of the apparent correlation between nitrogen content and the temperature coefficient for the second period is really due to the association of both with the temperature coefficient for the third period, and *vice versa*. When the sum of the temperature coefficients for the second and third periods was employed as a single independent variable, the resulting normal equations were

$$\begin{aligned} 196.8993b_0 - 10.6768b_{23} &= -13.3427 \\ -10.6768b_0 + 103.1914b_{23} &= 6.2085 \end{aligned}$$

and b_{23} assumed the more moderate value of $+0.053 \pm 0.022$. This is, however, still significantly greater than its standard error, and the degree of association with nitrogen content is measured by a partial correlation coefficient of $r = +0.33$, slightly in excess of the 5% point, 0.28. The value of b_0, the partial regression coefficient of nitrogen content on May and June rainfall after allowing for the temperature effect, is $-0.065 \pm 0.016\%$ nitrogen per additional inch of rain. The seasonal standard deviation of rainfall is approximately 1.4 times that of the sum of the two temperature coefficients, whereas the partial regression coefficient of nitrogen content on the combined temperature coefficients (b_{23}) is only about 0.8 of b_0, the partial regression coefficient on May and June rainfall. The temperature effects revealed by this analysis were thus a less potent source of variation in nitrogen content than were the fluctuations in rainfall.

In order to obtain a valid estimate of the relation for the two individual periods, the partial regression of nitrogen content on May and June rainfall and on the temperature coefficients for the second and third periods had to be determined simultaneously. The normal equations now became

$$\begin{aligned} 196.8993b_0 - 5.5323b'_2 - 5.1445b'_3 &= -13.3427 \\ -5.5323b_0 + 37.0529b'_2 + 13.1735b'_3 &= 3.4191 \\ -5.1445b_0 + 13.1735b'_2 + 39.7915b'_3 &= 2.7894 \end{aligned}$$

giving $b'_2 = +0.069 \pm 0.040$ and $b'_3 = 0.039 \pm 0.039$. Both coefficients are now less than twice their respective standard errors, indicating that the changes in nitrogen content associated with the temperature coefficient for a single period were not sufficiently pronounced to ensure their differentiation from the residual variation. The difference between b'_2 and b'_3 is also insignificant in comparison with its standard error, and cannot be regarded as plausibly suggesting a greater effect of temperature fluctuations in the second period.

Relation between Vegetative Growth, Yield and Nitrogen Content

In addition to the observations on yield and nitrogen content of grain already published (3, Table I) measurements of the average height of the plants were made at intervals throughout each growing season at each station. In so far as the final height attained provides an index of vegetative growth therefore, it was possible to investigate the relation between growth, yield and nitrogen content.

TABLE II

FINAL HEIGHT ATTAINED BY CROP (INCHES)

Station	Year	Height	Station	Year	Height
Lacombe, Alberta	1915	54	Indian Head, Saskatchewan	1928	49
	17	52		29	33
	21	46		31	26
	22	34		32	38
	25	43			
	28	55	Scott, Saskatchewan	1915	46
	30	38		17	26
	31	48		18	17
	32	54		21	34
				23	45
Lethbridge, Alberta	1915	48		24	20
	16	41		25	35
	17	33		26	28
	21	27		29	31
	22	36		30	34
	25	33		31	33
	26	36		32	36
	27	20			
	28	44	Rosthern, Saskatchewan	1915	39
	29	24		21	33
	30	32		22	48
	31	24		24	20
	32	33		25	41
				26	30
Indian Head, Saskatchewan	1915	48		27	48
	18	34		28	48
	24	38		29	27
	25	43		30	34
	26	44		32	32

The height attained by the various crops is shown in Table II. Seasonal variations gave rise to a standard deviation of 8.3 in., or 22.5% of the general mean, 36.9 in., and were therefore relatively less pronounced than the seasonal differences in yield and nitrogen content of grain, 41.8 and 29.6% of the mean respectively (3). The seasonal covariance of height and yield was computed to be 250387.6, and the sum of the squared deviations of the two quantities to be 3391.60 and 33,440,027, resulting in a coefficient of correlation of +0.74 (1% point = 0.36).

Previous calculations (3) had yielded a coefficient of correlation between nitrogen content and yield of $r = -0.40$. It was now found however that there was also an association between height and nitrogen content, giving a coefficient of $r = -0.50$; and when the coefficient of partial correlation between nitrogen content and yield after eliminating variations in both associated with height was determined, this was reduced to the negligible value of $r = -0.07$.

By the analysis described in the preceding paragraph, the seasonal variance of nitrogen content and yield was divided into two portions, of which one was associated with variations in height (*i.e.*, vegetative development). The other must be attributable to environmental factors not reflected in height or operative after elongation has ceased. This cessation occurs, under western Canadian conditions, about one week after flowering and from three to five weeks, depending on the season, before maturity. As the previously observed negative correlation between nitrogen content and yield arose from variations in these quantities associated with height differences, it would seem that any reductions in yield, due to restriction of the later stages of translocation, which occurred did not result in a significant modification of the nitrogen content of the grain.

General Conclusions

The results of this investigation as a whole are consistent with the supposition, inferred from chemical evidence in the introductory section of the present paper and stated explicitly by Russell and Bishop in connection with their studies on barley (7), that the nitrogen content of the grain is largely determined by conditions prevailing prior to the onset of translocation. This would certainly seem to be true of rainfall effects, and presumably of soil effects also.

On the other hand the views of McGinnis and Taylor with respect to the role of respiration (6) are also circumstantially supported, since the exponentially weighted temperature coefficients showed a significant correlation with nitrogen content whereas the linear temperature averages did not; and there remains the possibility that had more definite information respecting the temperature quotient of respiration been available, a more pronounced effect of temperature might have been demonstrated.

The theory that seasonal variations in moisture supply during the later weeks of development and ripening of the kernel are of prime importance in determining the composition of the grain is not, however, upheld by the present findings.

Acknowledgments

It is again a pleasure to acknowledge the co-operation of Dr. F. T. Shutt in making available for study his extensive series of field observations. Miss Mabel James also assisted largely in the arithmetical work.

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A STUDY OF MOISTURE CHANGES IN STANDING GRAIN[1]

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Abstract

A study of the moisture changes in standing grain during and after the ripening period was conducted at Winnipeg, Saskatoon and Edmonton in 1932 and 1933 with a view to obtaining information on the problem of combine harvesting.

Grain was found to be fit for binding four to seventeen days earlier than for straight combining.

There was no evidence that fully ripened grain at moisture contents of 11–13% can absorb sufficient moisture at night, owing to the higher relative humidity, to exceed 14·4% and become tough.

The rate of moisture loss in wet mature grain is much greater than the moisture loss, through the same range, in immature grain.

It is well known that wheat and other cereal grains lose moisture quite rapidly during maturation, but there is little definite information regarding the rate of loss under the climatic conditions encountered in Western Canada. Wheat that is ready for the binder usually contains from 20 to 35% moisture. In straight combining it is necessary to postpone harvesting until the moisture content has dropped to about 14% and the time necessary to bring this about is of considerable interest in relation to the choice of a method of harvesting.

Furthermore, in straight combining, cognizance must be taken of the possibility that during the night, when the relative humidity becomes high, the ripe dry grain may absorb enough moisture to make it tough in the morning, with the consequence that if operations are started too early in the morning or continued too late at night, the grain may carry an undesirable percentage of moisture. Numerous laboratory experiments have shown that the cereal grains are hygroscopic, and several studies have been conducted to determine the moisture content which will be attained when they are kept in atmospheres of different relative humidity. For example, Coleman and Fellows (1) found that at relative humidities of 15%, 45%, 75%, 90% and 100% the corresponding moistures of hard red spring wheat were 7·3%, 11·2%, 17·3%, 24·6% and 33·4% respectively. These are the percentages finally attained by the wheat, but it must be exposed six to eight days before these values are reached. For immature grain of high moisture content, the vapor pressure of the grain would exceed the partial pressure of water vapor in the air, even at the higher relative humidities during the night, and hence the grain would continue drying. However, as it matures it ultimately attains moisture contents at which its vapor pressure becomes lower than the

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partial pressure of water vapor in the air at the lower temperatures of the night. At this point it might be expected to lose moisture during the day and regain some of it at night. With grain bordering on "tough" this might become an important factor in straight combining. With these considerations in mind, an investigation was undertaken to determine the course of moisture variation in standing grain during its maturation period and also to ascertain the changes that might be expected to occur in fully ripened grain as a result of fluctuations in the relative humidity during 24-hr. periods. At Winnipeg, standing plots of Reward wheat and O.A.C. 21 barley were used. At Saskatoon and Edmonton, plots of Marquis wheat were sampled.

From approximately ten days after flowering, the plots were sampled at 2 p.m. daily until the grain reached a moisture content of about 45%. From that stage until the grain was dead ripe, sampling was done at 2-hr. intervals from 8 a.m. to 8 p.m. inclusive in 1932, and from 6 a.m. to 10 p.m. inclusive in 1933. During the 1932 experiments, heads were taken at random over the whole plot, transferred to air-tight containers, threshed either by hand or in a small head-thresher, and tested for moisture immediately on returning to the laboratory. In 1933 a modification of the method of sampling was used. The plots were divided into sections, which were numbered and randomized; all the heads in a given section were taken and duplicate samples were collected at each sampling period. Until the moisture reached 15%, the determinations were made in two stages. A large sample of 30 to 40 gm. was given a first drying by heating in an air oven at 98° C. for 5 hr. Moisture loss on this was determined, the grain was then ground and the residual moisture determined on a 2 gm. sample in the usual way.

Records of temperature and relative humidity were made either at each reading or by means of a recording instrument. Average wind velocity, hours of sunshine and rainfall were obtained from the meteorological records (a Class I station is located at each point). At Winnipeg a record was kept of the daily evaporation.

The complete data are too bulky for inclusion in this paper, therefore only the summary of the moisture results, together with graphs showing moisture, temperature, humidity, sunshine, rainfall and wind velocity are presented.

As this work comprises three separate and distinct experiments, each carried on under different climatic conditions in two successive seasons, they will be discussed separately.

Rate of Drying of Barley at Winnipeg, 1932

Sampling of the barley plot at Winnipeg began on July 30, 1932, the moisture content of the grain at that time being 60·3%. One sample per day was taken until Aug. 5, by which time the moisture had fallen to 48·2% and thereafter sampling was carried on at 2-hr. intervals from 8 a.m. to 8 p.m. daily until Aug. 15, when the moisture had fallen to below 13%. The data are given in Table I and shown graphically in Fig. 1. The meteorological data for this period are given in Table XIII. This period at Winnipeg was characterized by gradually increasing temperature during the day, long duration of bright sunshine, moderate wind velocities and little rain; almost ideal conditions for studying rate of desiccation of maturing grain in normal hot dry weather.

TABLE I

MOISTURE CONTENT OF STANDING BARLEY AT 2-HR. INTERVALS FROM 8:00 A.M. TO 8:00 P.M. DURING RIPENING PERIOD AT WINNIPEG, 1932

Date	Moisture content at							Daily mean, %
	8 a.m. %	10 a.m. %	12 a.m. %	2 p.m. %	4 p.m. %	6 p.m. %	8 p.m. %	
Aug. 6	48.7	48.0	48.9	48.3	46.5	43.9	46.4	47.2
7	46.3	45.5	46.0	45.0	45.8	45.8	44.6	45.6
8	45.4	43.7	43.9	43.6	43.7	43.1	43.4	43.8
9	42.5	41.8	41.5	40.5	39.2	40.6	40.8	41.0
10	37.7	38.4	41.0	38.8	34.9	34.8	37.4	37.6
11	36.4	36.3	34.8	34.2	30.6	32.8	31.9	33.9
12	34.0	31.0	29.7	28.0	29.0	31.3	25.2	29.7
13	28.0	28.2	25.9	25.8	25.2	26.7	25.8	26.5
14	22.1	22.9	21.6	20.7	20.6	21.0	17.9	21.0
15	16.0	13.3	13.8	11.1	11.6	12.1	14.4	13.2
16	15.9	14.9	15.0	14.3	13.4	12.0	13.0	

	10 p.m.	12 p.m.	2 a.m.	4 a.m.	6 a.m.
Aug. 15 and Aug. 16	14.5	16.5	16.7	16.2	16.2

It will be noted in Fig. I that the moisture content fell rather slowly from about 48% on August 6 to 36% at 8 a.m. August 11, and thereafter more rapidly until at midday August 15 it had decreased to 14.3%. This was obviously the earliest date at which combining might be started if straight grade grain were desired. This grain, however, was judged fit for cutting with the binder on August 11. At that time the moisture was between 31 and 34%. It would have been necessary, therefore, to wait four days after binding started to commence combining, even with the ideal weather conditions that prevailed during this period.

There seems to have been no pronounced rise in moisture content during the night until the grain decreased to about 12% moisture. The rise indicated on the morning of August 13 was probably due to sampling error, because the second and third samples for that day rose successively, although the temperature had risen as usual quite sharply. To get more accurate information concerning the change in moisture content of the grain during the night, the barley plot was sampled at 2-hr. intervals from 8 a.m. August 15 to 8 p.m August 16. From a moisture content of 16% at 8 a.m. the grain dried to 11.1% at 2 p.m. and then increased to 14.4% at 8 p.m. the same evening. The maximum moisture, 16.7%, was recorded at 2 a.m. the following morning. This fell to 15.9% at 8 a.m., an increase of 1.5% over the value obtained at 8 p.m. the previous evening. The increase is not great, but it is of significance in relation to the time at which combines may resume work.

Rate of Drying of Reward Wheat at Winnipeg, 1932

The data on moisture in the wheat samples are given in Table II and shown graphically in Fig. 1.

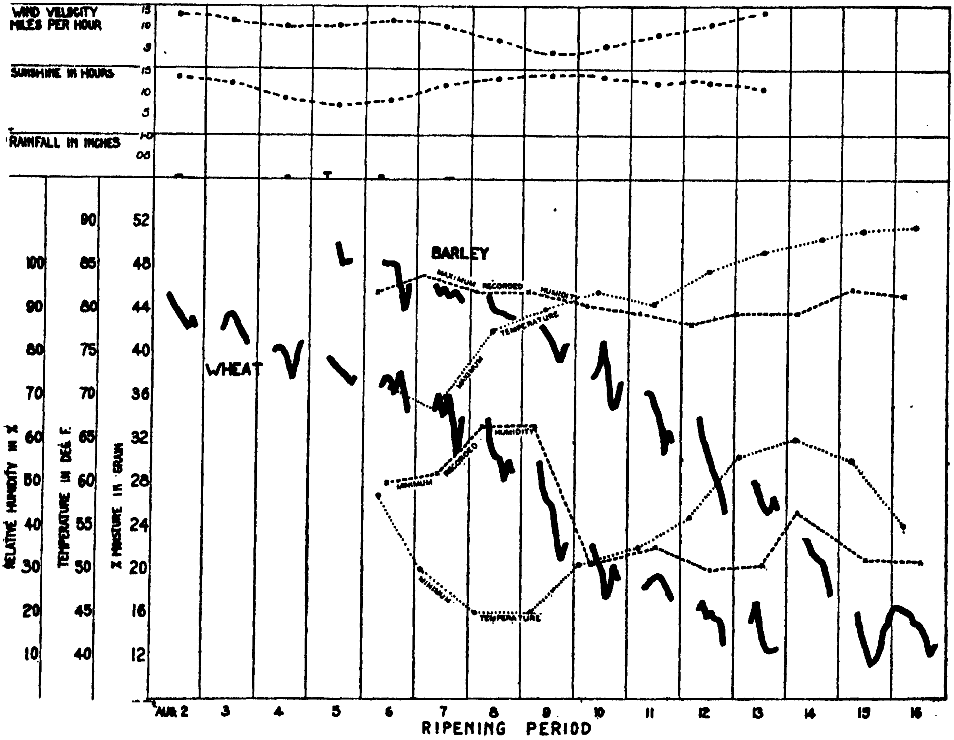

FIG. 1. *Changes in moisture content of Reward wheat and O.A.C. 21 barley, and weather conditions at Winnipeg, 1932.*

TABLE II

MOISTURE CONTENT OF STANDING WHEAT AT 2-HR. INTERVALS FROM 8:00 A.M. TO 8:00 P.M. DURING RIPENING PERIOD AT WINNIPEG, 1932

Date	Moisture content at							Daily mean, %
	8 a.m. %	10 a.m. %	12 a.m. %	2 p.m. %	4 p.m. %	6 p.m. %	8 p.m. %	
Aug. 2	45.4	43.5	44.6	42.9	42.0	43.2	42.3	43.4
3	42.0	43.3	43.5	—	—	—	40.7	42.4
4	40.0	40.5	40.1	39.0	37.6	40.5	40.9	39.8
5	39.5	38.4	39.2	37.0	38.2	36.8	37.5	38.1
6	36.8	37.8	37.5	35.9	38.2	36.4	34.3	36.7
7	34.9	36.1	33.9	36.0	34.9	30.6	33.9	34.3
8	33.8	30.9	30.2	30.2	28.0	30.0	29.0	30.3
9	29.9	25.9	26.7	26.0	22.0	20.8	22.3	24.8
10	22.2	20.8	20.2	17.3	17.7	20.3	19.0	19.6
11	18.2	18.8	19.2	19.3	19.0	—	17.2	18.6
12	16.3	17.0	15.5	16.1	15.4	15.1	13.0	15.5
13	15.1	16.9	14.6	12.9	12.4	12.5	12.8	13.9

Sampling of wheat at 2-hr. intervals was commenced at 8 a.m. August 2 and continued until 8 p.m. August 13. The grain was judged fit for cutting with the binder on August 6. At this date the moisture content was about 36%. It was not under 14.4% until 2 p.m. August 13. This means that combine harvesting could not have been started until seven days after binder cutting.

In this series there is no pronounced evidence of increase in moisture content during the night. At 8 p.m. August 12 the moisture was 13.0%, having dropped from 15.1% at 6 p.m. that day; the following morning at 8 a.m. it was 15.1% which looks like an increase due to higher humidity during the night. It appears probable, however, that the value 13% ought to be considered an error due to sampling.

It is interesting to note that the standing barley took eight days to dry from 45% to 12% moisture, while the Reward wheat required 12 days for the same change. This is accounted for by the fact that light showers occurred on all but one day during the period August 2–7 inclusive. Even though the showers were slight and generally of short duration, as can be seen from the hours of bright sunshine, and there were moderate winds, these rains effectively retarded drying to the extent that during these six days the wheat changed only from 45.4 to 33.8% moisture, whereas during the next six days it changed from 33.8 to 12.8%.

Rate of Drying of Barley at Winnipeg, 1933

In 1933 barley only was used at Winnipeg. Sampling at 2-hr. intervals commenced on August 9 and continued until August 27. The average

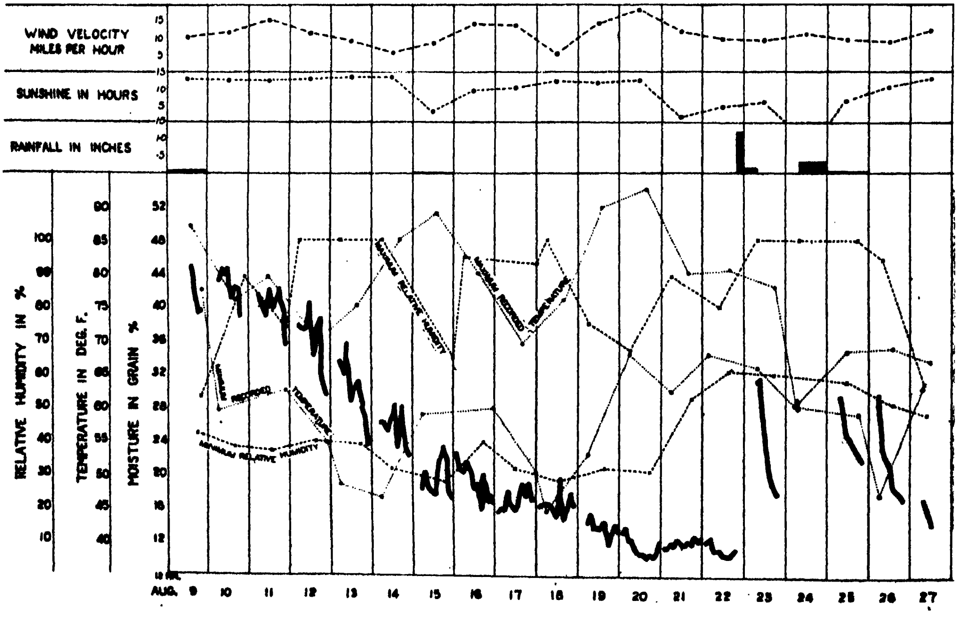

FIG. 2. *Changes in moisture content of O.A.C. barley, and weather conditions at Winnipeg, 1933.*

moisture for each sampling is given in Table III and shown graphically in Fig. 2. The meteorological data are given in Table XIV.

TABLE III

MOISTURE CONTENT OF BARLEY IN STANDING GRAIN AT 2-HR. INTERVALS FROM 2:00 A.M. TO 12:00 P.M. DURING RIPENING PERIOD AT WINNIPEG

Date	Moisture content at												Daily mean, %
	2 a.m. %	4 a.m. %	6 a.m. %	8 a.m. %	10a.m. %	12 noon %	2 p.m. %	4 p.m. %	6 p.m. %	8 p.m. %	10p.m. %	12p.m. %	
Aug. 9	—	—	—	—	—	—	44.9	42.0	39.2	39.3	—	—	41.4
10	—	—	43.0	44.6	43.8	44.7	40.9	42.4	42.2	38.8	—	—	42.6
11	—	—	41.6	40.2	38.6	42.1	39.8	40.8	42.3	39.6	35.4		40.0
12	—	—	37.9	37.4	37.4	40.6	34.0	37.6	38.8	31.0	29.3	—	36.0
13	—	—	33.7	31.8	35.8	28.6	30.2	31.2	28.0	27.3	23.4	—	30.0
14	—	—	26.3	26.3	25.2	26.9	28.4	23.1	28.3	23.2	22.2	—	25.5
15	—	—	18.2	20.3	18.4	17.8	17.5	21.9	23.4	21.9	18.0	16.8	19.4
16	22.6	20.7	20.2	21.7	21.2	18.2	19.1	15.8	20.0	16.6	17.6	16.6	19.2
17	15.5	16.1	15.7	18.1	16.4	15.5	16.4	18.9	17.3	19.0	16.9	17.0	16.9
18	16.2	16.4	16.6	16.6	16.1	14.8	19.7	14.6	15.5	17.8	16.2	—	16.5
19	—	—	14.2	15.4	13.6	13.6	13.6	14.4	11.5	12.8	14.1	12.8	13.6
20	13.1	13.8	12.2	12.3	11.0	10.6	10.6	10.0	10.6	10.0	10.6	12.2	11.4
21	11.3	11.3	11.8	11.4	12.4	11.6	11.5	12.1	11.6	12.7	12.0	12.6	11.9
22	12.0	12.2	12.5	10.8	11.0	10.4	10.2	10.2	10.6	11.1	Rain		11.1
23	Rain			31.2	31.5	25.8	21.4	—	18.2	17.5	—	—	24.3
24	Rain		29.0	28.0	Rain								28.5
25	—	—	—	29.4	27.3	24.6	24.1	23.1	22.4	21.6	—	—	24.6
26	—	—	29.6	24.2	22.0	21.2	18.6	18.4	17.6	17.0	—	—	21.1
27	—	—	—	17.0	15.6	14.0	—	—	—	—	—	—	15.5

Beginning on August 9 with a moisture content of 44.9%, the grain dried rapidly until August 13, when it reached an average daily moisture content of 30% and was fit for binder cutting. It required six days longer, or until August 19, before it would have been harvested safely by straight combining. Between 8 p.m. August 22 and 6 a.m. August 23, precipitation to the amount of 1.22 in. occurred. This caused an abrupt rise in moisture content to 31%. Showers during the next three days prevented rapid drying and it was not until noon, August 27, that the grain on this plot contained less than 14.4% moisture and could be considered fit for straight combining.

Rate of Drying of Marquis Wheat at Saskatoon

In 1932, sampling of the wheat plot at Saskatoon began on August 4 when the moisture content of the grain was 40%. The grain was judged fit for binding on the afternoon of August 5, at which time the moisture content was 33–34%. By the afternoon of August 9 this had decreased to less than 14.4% and the grain was fit for straight combining. The rate of drying was rapid, owing to hot dry days with moderate winds and long hours of bright sunshine. Sampling was continued until August 27 in order to observe the effect of rains. The data are given in Table IV and shown graphically in Fig. 3.

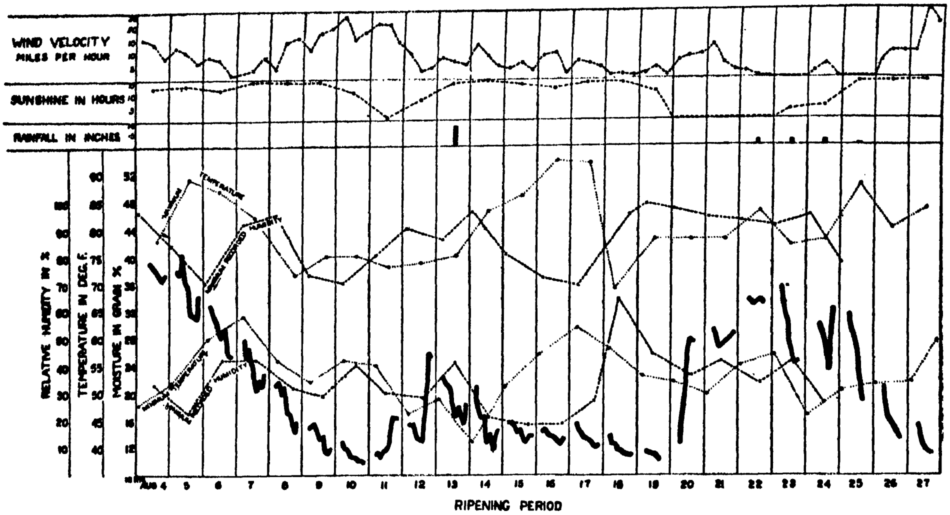

FIG. 3. *Changes in moisture content of Marquis wheat, and weather conditions at Saskatoon, 1932.*

TABLE IV

MOISTURE CONTENT OF STANDING MARQUIS WHEAT AT 2-HR. INTERVALS FROM 5:00 A.M. TO 9:00 P.M. DURING RIPENING PERIOD AT SASKATOON, 1932

Date	Moisture content at									Daily mean, %
	6 a.m. %	8 a.m. %	10 a.m. %	12 noon %	2 p.m. %	4 p.m. %	6 p.m. %	8 p.m. %	10 p.m. %	
Aug. 4	—	—	39.3	—	—	37.7	37.3	36.3	37.2	37.6
5	37.8	37.3	40.5	36.7	36.3	31.6	31.2	30.8	34.3	35.2
6	32.9	31.9	31.4	30.4	27.9	29.8	28.3	25.7	25.4	29.3
7	27.9	25.0	26.8	25.4	22.2	20.1	22.0	20.7	22.8	23.6
8	21.1	21.8	19.5	20.9	17.2	17.3	15.3	14.1	15.8	18.1
9	15.0	15.5	15.7	15.3	13.1	13.4	11.4	11.0	11.9	13.6
10	12.8	11.8	11.9	11.1	10.6	10.8	10.0	10.1	9.9	11.0
11	11.2	11.3	10.4	11.7	11.8	12.3	16.4	16.5	16.1	13.1
12	15.5	15.3	15.7	13.5	13.3	12.7	—	25.9	25.3	17.1
13	22.4	21.4	21.2	20.5	17.6	16.3	18.0	15.2	18.2	19.0
14	20.9	17.4	16.2	16.7	12.7	14.0	11.2	13.8	14.6	15.3
15	15.9	15.8	15.0	14.3	14.6	13.4	12.8	13.8	13.8	14.4
16	14.4	14.7	13.9	13.7	13.7	13.2	12.7	12.6	13.4	13.6
17	15.6	14.3	14.2	13.3	13.3	12.8	12.1	11.9	12.3	13.3
18	13.7	12.5	12.5	11.7	11.7	11.1	11.1	11.0	11.1	11.8
19	—	11.6	—	—	11.0	—	10.1	—	—	10.9
20	—	12.5	—	—	27.8	—	27.5	—	—	22.9
21	—	29.2	—	26.6	—	27.3	—	—	28.8	28.0
22	—	33.7	—	32.8	—	33.7	—	33.0	—	33.3
23	—	35.5	—	30.5	27.5	—	24.1	24.4	—	28.4
24	—	—	29.9	—	28.2	26.5	23.2	32.2	—	28.0
25	—	31.4	29.2	27.5	23.7	21.7	18.6	—	—	25.4
26	—	20.9	18.0	16.5	16.1	15.4	14.0	13.3	—	16.3
27	—	15.3	14.2	12.9	12.1	11.4	11.2	—	—	12.9

In 1933 sampling of a Marquis wheat plot was commenced on August 1, when the moisture content of the wheat was 40%. The grain was judged fit for binding on August 4, when the moisture had fallen to about 32–33%.

Six days later, August 10, the moisture content was less than 14.4% and the wheat was fit for straight combining. Then followed a period of eleven days, August 10–20 inclusive, of ideal harvest weather, during which there were uniformly long "hours of bright sunlight", light winds, high daily temperatures, a very uniform range of relative humidity, and only one slight shower of rain. Thereafter the weather became somewhat unsettled with cloudy days and occasional rains. The sampling was continued until September 25, in order to observe the rate of drying in the latter part of the harvest season. The moisture data are given in Table V and in Fig. 4. The meteorological data are given in Table XVI.

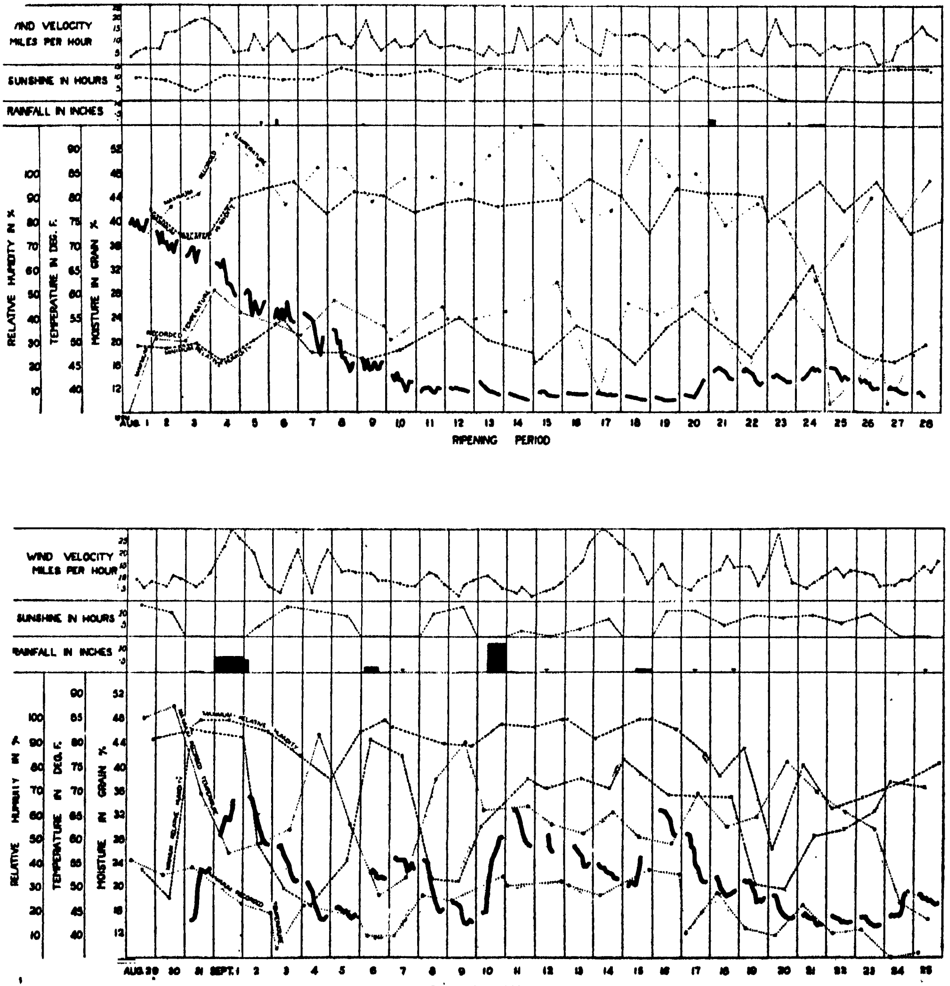

FIG. 4. *Changes in moisture content of Marquis wheat, and weather conditions at Saskatoon, 1933.*

TABLE V

MOISTURE CONTENT OF STANDING MARQUIS WHEAT AT 2-HR. INTERVALS FROM 5:00 A.M. TO 9:00 P.M. DURING RIPENING PERIOD AT SASKATOON, 1933

Date	Moisture content at									Daily mean, %
	6 a.m. %	8 a.m. %	10 a.m. %	12 noon %	2 p.m. %	4 p.m. %	6 p.m. %	8 p.m. %	10 p.m. %	
Aug. 1	—	39.6	40.6	39.0	40.1	38.6	39.0	38.3	40.2	39.4
2	38.2	36.2	38.0	36.4	36.5	35.2	36.2	34.8	36.5	36.4
3	34.1	34.3	35.8	35.7	33.1	35.0	—	—	—	34.7
4	33.1	33.0	32.3	33.7	31.9	29.8	29.4	28.2	27.3	31.0
5	27.9	28.5	27.5	24.2	26.7	25.3	24.3	—	26.3	26.3
6	23.9	25.5	23.8	25.4	23.7	26.4	24.6	23.2	22.8	24.4
7	24.6	24.2	23.8	23.5	23.3	20.7	18.9	17.5	20.5	21.9
8	21.9	21.7	19 6	19.9	17.2	17.1	15.8	14.8	16.0	18.2
9	17.2	14.9	16.4	14.9	15.2	16.4	15.1	15.4	16.4	15.8
10	14.1	13.1	14.6	13.2	13.2	12.6	11.5	13.0	13.0	13.1
11	11.6	12.0	12.2	12.3	12.1	11.4	11.3	12.2	11.8	11.9
12	12.0	11.8	12.1	12.0	—	—	—	—	11.6	11.9
13	13.1	—	—	—	11.5	—	—	—	10.8	11.8
14	11.1	—	—	—	10.5	—	—	—	9.9	10.5
15	11.3	11.5	11.5	11.3	10.9	—	—	—	10.6	11.2
16	11.2	—	—	—	11.0	—	—	—	11.1	11.1
17	11.2	—	—	—	10.8	—	—	—	10.8	10.9
18	10.6	—	—	—	10.3	—	—	—	10.1	10.3
19	10.3	—	—	—	9.9	—	—	—	10.0	10.1
20	10.8	—	—	—	10.4	—	—	—	13.4	11.5
21	14.8	15.4	15.6	15.3	15.2	14.7	13.9	13.6	13.5	14.7
22	14.8	15.3	14.9	14.6	14.3	13.3	13.0	12.5	12.8	13.9
23	13.9	13.9	14.2	14.0	13.6	13.5	13.0	13.0	13.0	13.6
24	13.3	13.5	13.9	14.7	15.2	15.4	14.9	15.1	14.7	14.5
25	15.3	15.3	15.3	14.8	14.2	13.2	13.9	13.5	13.5	14.3
26	13.6	13.0	13.4	12.9	12.9	12.2	11.7	11.9	11.9	12.6
27	11.9	12.3	11.9	11.9	11.7	11.3	11.1	11.0	11.1	11.6
28	11.2	11.4	10.9	10.6	—	—	—	—	—	11.0
29	—	—	—	—	10.9	—	—	—	—	10.9
30	—	—	—	—	11.1	—	—	—	—	11.1
31	14.4	14.4	15.2	—	23.0	22.7	22.4	22.5	23.1	19.7
Sept. 1	28.5	30.6	31.8	—	31.2	34.5	—	—	—	31.3
2	35.2	34.8	33.8	31.9	30.9	29.0	27.9	27.2	27.1	31.0
3	26.8	27.1	26.1	25.2	24.1	22.6	21.6	20.9	20.9	23.9
4	20.7	19.9	19.4	17.4	16.4	14.8	14.3	14.2	14.9	16.9
5	16.3	16.5	16.2	15.7	15.8	15.0	14.8	14.6	15.2	15.6
6	—	—	22.2	22.8	22.2	21.4	21.6	21.5	21.2	21.8
7	24.7	24.4	24.4	24.4	24.3	22.4	22.5	23.8	22.7	23.7
8	24.5	24.3	23.9	20.4	19.4	17.9	16.1	15.7	16.1	19.8
9	17.2	17.2	16.8	16.8	15.2	13.8	13.5	14.4	13.9	15.4
10	15.5	15.5	19.1	22.5	24.9	25.9	27.0	28.1	28.2	23.1
11	—	32.7	32.9	31.9	30.3	28.4	27.8	27.1	26.7	28.4
12	—	—	—	28.5	26.4	25.6	—	—	—	26.8
13	—	—	26.7	26.3	25.5	25.1	23.3	23.2	23.7	24.8
14	23.6	23.2	23.1	22.4	22.2	22.0	21.3	21.3	21.2	22.3
15	19.9	20.8	20.3	20.1	22.8	24.9	—	—	—	21.5
16	—	32.7	—	32.6	—	31.5	—	28.4	—	31.3
17	28.8	28.6	26.8	26.5	23.4	22.6	21.0	20.7	20.8	24.4
18	21.4	21.5	19.8	19.2	18.3	18.2	18.3	18.7	19.0	19.4

The Rate of Drying of Marquis Wheat at Edmonton

Sampling of the Marquis wheat plot at Edmonton in 1932 was commenced on August 23, when the moisture content of the wheat was 48%. On August 29 the daily average moisture content had fallen to 34% and the grain was ready for binding. After that, the rate of drying was slow, owing to frequent light showers, and it was not until September 11, 13 days later, that the moisture content was less than 14.4% so that the grain could be considered fit for straight combining. Sampling was continued until September 14. The moisture data are given in Table VI and in Fig. 5; the meteorological data are to be found in Table XVII.

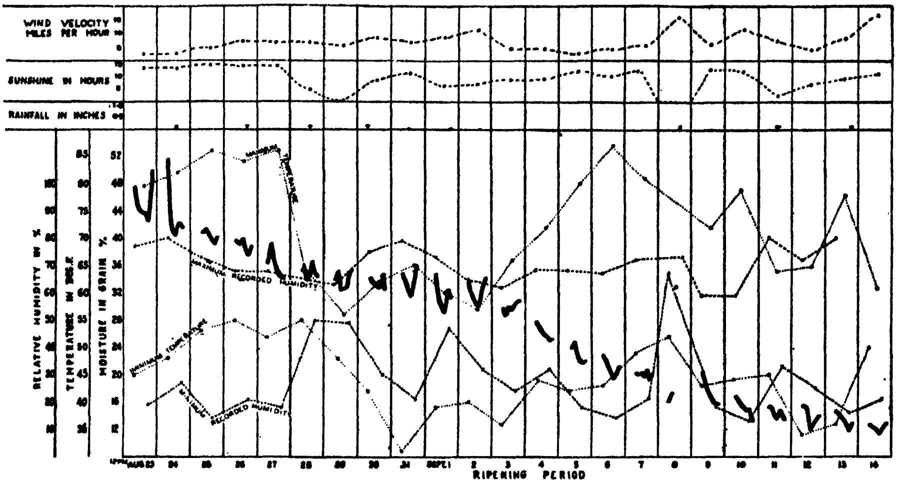

FIG. 5. *Changes in moisture content of Marquis wheat, and weather conditions at Edmonton, 1932.*

TABLE VI

MOISTURE CONTENT OF STANDING MARQUIS WHEAT AT 2-HR. INTERVALS FROM 8:00 A.M. TO 8:00 P.M. DURING RIPENING PERIOD AT EDMONTON, 1932

Date	Moisture content at							Daily mean, %
	8 a.m. %	10 a.m. %	12 a.m. %	2 p.m. %	4 p.m. %	6 p.m. %	8 p.m. %	
Aug. 23	47.5	44.4	43.8	43.6	43.7	42.5	49.9	45.0
24	51.6	42.8	40.3	40.4	42.4	41.4	42.1	43.0
25	40.8	40.8	41.4	40.0	40.7	39.0	40.2	40.4
26	39.7	39.5	39.0	39.9	37.9	37.5	38.9	38.9
27	36.2	39.3	37.4	34.9	34.9	34.5	34.1	35.9
28	35.1	35.9	33.7	34.9	36.6	34.9	34.4	35.1
29	33.8	35.3	32.2	33.0	32.6	34.9	35.4	33.9
30	33.8	34.3	33.0	33.5	32.6	34.7	32.4	33.5
31	34.8	33.4	33.4	31.4	32.0	36.0	—	33.5
Sept. 1	34.7	31.0	31.0	29.0	32.7	31.1	31.6	31.6
2	33.5	29.5	31.9	30.0	30.7	33.2	34.2	31.8
3	29.8	28.4	30.1	29.4	29.9	30.6	30.7	29.8
4	27.8	26.7	25.9	25.9	26.2	24.9	25.2	26.1
5	31.8	23.9	24.9	22.0	22.4	21.6	22.0	24.1
6	22.9	22.0	21.2	19.9	19.3	20.3	21.2	21.0
7	20.2	19.9	20.3	19.4	20.7	19.0	20.0	19.6
8	15.9	17.1			Rain			
9	20.4	17.9	17.9	17.2	15.6	16.1	15.7	17.2
10	16.8	16.2	15.4	15.2	14.6	15.0	13.0	15.2
11	15.3	15.2	13.8	13.4	14.8	14.2	13.3	14.3
12	15.4	15.4	14.7	11.8	12.8	13.7	14.0	14.0
13	14.7	14.2	14.0	13.4	13.2	11.7	12.9	13.4
14	12.7	12.1	12.7	11.7	11.4	12.3	12.9	12.2

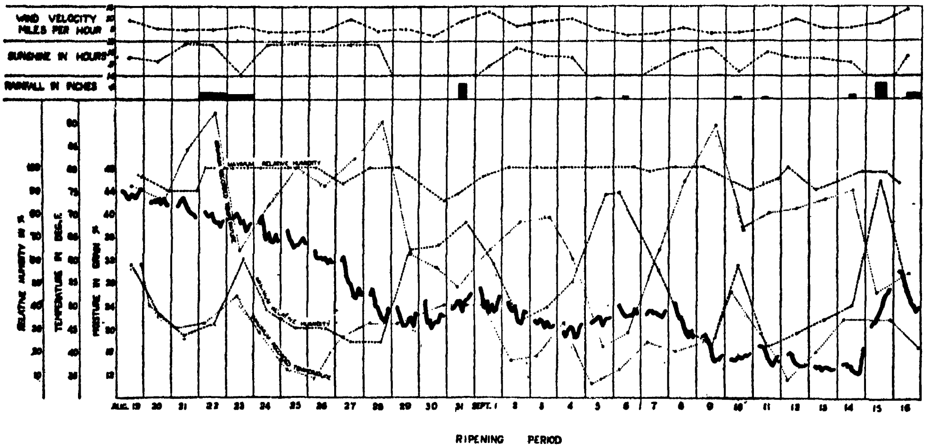

FIG. 6. *Changes in moisture content of Marquis wheat, and weather conditions at Edmonton, 1933.*

TABLE VII

MOISTURE CONTENT OF STANDING MARQUIS WHEAT AT 2-HR. INTERVALS FROM 6:00 A.M. TO 10:00 P.M. DURING RIPENING PERIOD AT EDMONTON, 1933

Date	Moisture content at									Daily average, %
	6 a.m. %	8 a.m. %	10 a.m. %	12 noon %	2 p.m. %	4 p.m. %	6 p.m. %	8 p.m. %	10 p.m. %	
Aug. 19	44	43.8	42.9	42.6	43.4	42.9	42.7	43.4	44.3	43.3
20	42	41.9	42.3	42	42.7	41.7	42.7	41.6	41.4	42.0
21	41.35	42.3	42.9	42	41.3	40.5	40	39.75	39.3	41.0
22	40.2	39.85	39.4	40.1	38.8	38.3	38.6	37.5	38.8	39.1
23	39.2	39.5	40.2	39.5	38.25	38.9	36.7	37.25	38.2	38.6
24	38.4	39.6	37.8	35.4	36.7	35	34.9	36.4	35.05	36.6
25	37.15	35.65	35.15	33.9	34.6	34.45	35.15	35.75	34.7	35.2
26	33.65	32.3	32.1	32.35	32.05	32.35	31.25	32.05	31.3	32.2
27	31.8	32.45	30.05	27.95	27.75	25.45	25	26.95	25.6	28.1
28	25.75	26.8	25.3	23.75	23.65	21.9	21.55	20.95	23.5	23.7
29	23.7	22.35	21.35	20.25	20.3	21.55	21.9	20.25	22.65	21.6
30	24.8	20.8	21.4	19.75	20.75	21.1	20.8	22.4	22.15	21.6
31	23.6	23.8	24.75	24.7	22.8	24.7	25.15	25.6	26.05	24.6
Sept. 1	27	25.5	23.35	24.7	22.55	24.15	22.7	23.2	25.65	24.3
2	24.45	23.65	22.8	23.8	21.45	20.7	20.65	22.95	22.7	22.6
3	21.3	20.65	21.55	21.25	20	21.25	20.15	20.1	20.55	20.8
4	19.35	18.45	19.55	20.3	19.8	18.25	18.55	20	20.75	19.4
5	20.95	21.2	21.5	22.0	21.9	20.25	21.6	21.85	21.85	21.5
6	23.95	23.15	22.4	21.9	21.95	22.35	22	23.4	22.55	22.6
7	22.55	23.05	23.05	22.7	22.7	22.55	22.15	21.95	23	22.6
8	24.3	23.05	21.7	21.95	20.25	19.25	18.4	19.6	18.35	20.8
9	18.85	19.05	17.45	17.1	15.25	14.25	14.7	14.65	15.35	16.3
10	14.75	14.6	14.5	15.3	14.85	14.7	15.45	15.2	15.65	15.0
11	17.15	16.8	16.5	15.4	14.75	14.2	13.55	14.3	15.55	15.4
12	15.65	15.9	15.55	14.15	13.8	13.7	13.55	13.55	13.7	14.4
13	13.6	13.3	13.6	12.8	13.6	13.55	13.25	13.1	13.15	13.3
14	14	14.05	13.65	13.75	13.05	12.5	12.35	12.35	16.8	13.6
15	20.4	21.25	21.35	21.9	23.2	24.7	25.9	26.4	27	23.6
16	30.5	29.8	28.75	26.7	25.6	24.35	24.15	22.95	23.75	25.3

The rate of drying at Edmonton in 1933 proved to be even slower than in 1932, owing to the occurrence of several fairly heavy rains. Sampling was begun on August 19, when the moisture content was 44%. By midday August 25, the moisture content had fallen to about 34% and the grain was fit for binding. By 4 p.m. September 11 the wheat was dry enough for straight combining. It required 17 days from the time when binder harvesting could have been started for the grain to reach the straight grade moisture level. Sampling was continued until September 16. The moisture data are given in Table VII and the meteorological data in Table XVIII. Both are shown graphically in Fig. 6.

Comparison of Rates of Drying

The foregoing brief discussion of the separate investigations of rates of drying of grain may be summarized for the purpose of comparison by tabulating, as in Table VIII, the times that elapsed between the dates on which the grain was ready for binder harvesting and those on which it had dried to below 14.4%, the upper limit for safe straight combining.

TABLE VIII

DATES ON WHICH BINDER HARVESTING AND STRAIGHT COMBINING HARVESTING COULD HAVE BEEN STARTED

Place and year	Grain	First date on which moisture content had fallen to below		Difference in days A to B
		A—35%	B—14.4%	
Winnipeg, 1932	Barley	Aug. 11	Aug. 15	4
Winnipeg, 1932	Reward wheat	Aug. 7	Aug. 12	5
Winnipeg, 1933	Barley	Aug. 12	Aug. 19	7
Saskatoon, 1932	Marquis wheat	Aug. 5	Aug. 9	4
Saskatoon, 1933	Marquis wheat	Aug. 3	Aug. 10	7
Edmonton, 1932	Marquis wheat	Aug. 28	Sept. 10	13
Edmonton, 1933	Marquis wheat	Aug. 25	Sept. 11	17

The course of the drying of standing grain at the three places is shown graphically in Fig. 7. The principal fact brought out by this presentation of the data is that in the two seasons under consideration the rate of drying of wheat from 35% to 14.4% moisture content was two to three times faster at Winnipeg and Saskatoon than at Edmonton. It should be noted that at Edmonton in each year binder harvesting was 22–23 days later than at Saskatoon, while straight-combine harvesting would have been 31–32 days later. The normal expectancy of favorable harvest weather at the three points is greater for August than for September and therefore the delay of about two weeks between binder harvesting and combine harvesting at Edmonton must be regarded as very hazardous.

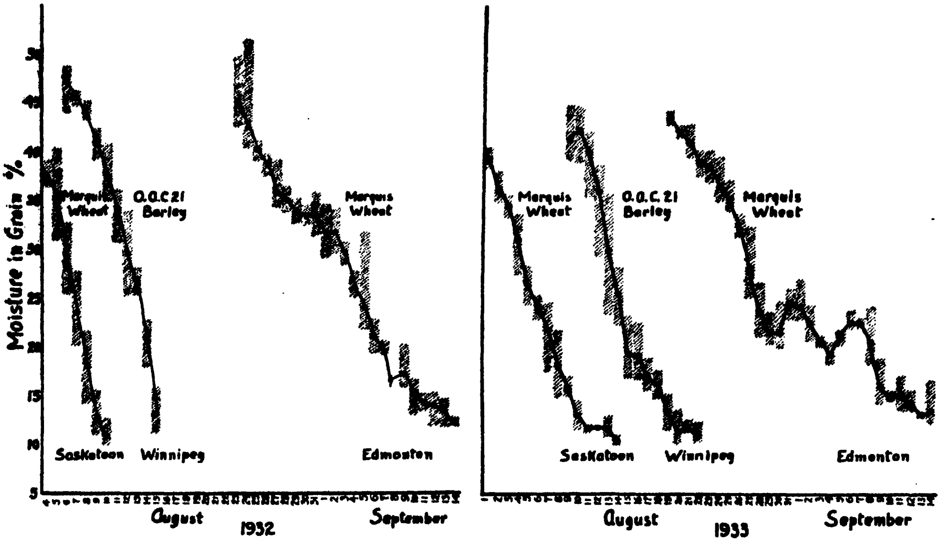

FIG. 7. *Comparison of rates of drying of standing grain at Winnipeg, Saskatoon and Edmonton.*

The Relation of Relative Humidity to Moisture of Standing Grain

It is commonly observed by combine operators that toward the end of the day and very early in the day, grain becomes too "tough" to handle properly. This is sometimes interpreted as meaning that the moisture content of the wheat increases to above 14.4%, the upper limit for straight grade wheat. It can be easily verified that the straw and more particularly the rachis frequently loses its brittleness late in the day, even during the most ideal harvesting weather. This is obviously due to the fall in temperature and the accompanying rise in relative humidity, which results in absorption of water vapor from the air by the straw. While no work has been reported on the relation of relative humidity to brittleness of straw, it seems reasonably certain from practical observation that the change takes place fairly rapidly.

The establishing of equilibrium between moisture content of grain and the humidity of the surrounding atmosphere is known to be a slow process, requiring from six to eight days when the air is circulated and from ten to twenty days in still air. Under the conditions of constantly changing relative humidity of a clear summer night, however, it is difficult to predict to what extent the moisture content of grain initially at 13–14% might increase. Laboratory experiments by one of the authors show that wheat at 11% moisture content, when exposed over water in a closed vessel, requires practically 12 hours to attain a moisture content of 14.4%. At lower relative humidities the rate of moisture increase is slower. From the practical point of view it is necessary to ascertain, if possible, whether or not wheat below 14.4% moisture may become tough over night, as a result of the increase in relative humidity. Part of the data obtained in this series of observations is suitable for study of this question.

In the Winnipeg 1933 data in Table III, there are 89 determinations of moisture of standing barley taken at consecutive 2-hr. intervals, covering the period 6 a.m. Aug. 15 to 10 p.m. Aug. 22, except for three samplings omitted at 12 p.m., 2 a.m. and 4 a.m. on the night of August 18. This period represented almost ideal harvesting weather, as can be seen from the meteorological data in Table XIV. The weather was mostly clear, with moderate winds, fairly high temperatures, long hours of sunshine and no rain except 0.02 in. on the first day, August 15. The moisture and relative humidity data for this period are shown in Fig. 8. The range of moisture represented by the duplicate samplings is shown by shaded columns and the averages by dots connected by the heavy line.

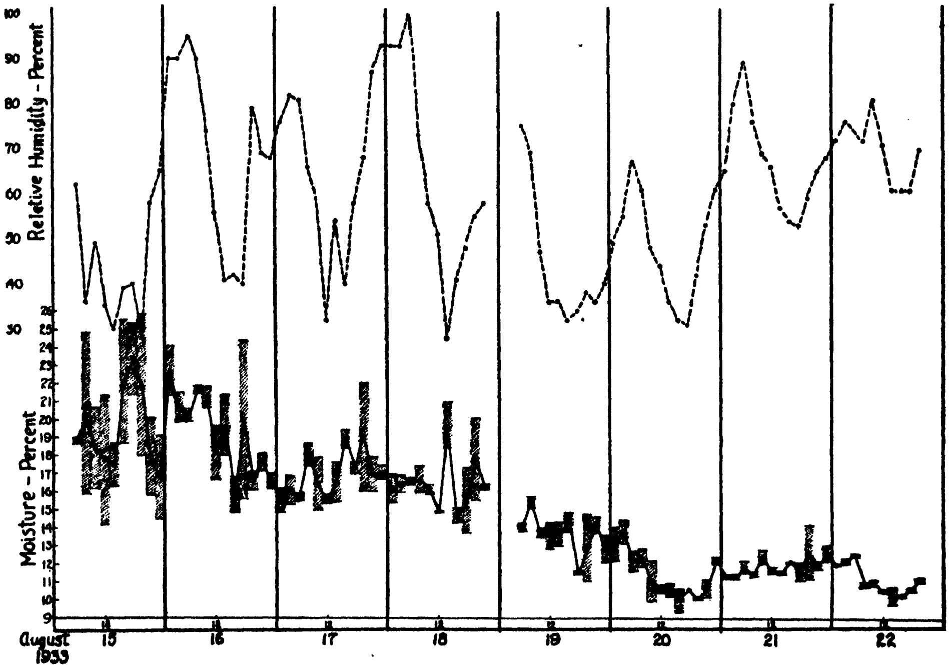

FIG. 8. *Upper and lower limits and average of moisture content of wheat samples collected at 2-hr. intervals for eight consecutive days at Winnipeg in 1933.*

The relative humidity for the first six days approached an observed minimum of about 30% in the early afternoon and an observed maximum of 80–100% in the early morning at 4 a.m. or 6 a.m. The changes in relative humidity were rapid, both minimum and maximum values being maintained for only short times. Recording instruments show that this is quite characteristic during clear settled weather in Western Canada. There appears to be some regional difference in the time at which the maximum is reached. For example, at Saskatoon the highest relative humidity during August occurs usually at about 2 a.m., apparently about two or three hours earlier than at Winnipeg.

At the beginning of the period under discussion, the moisture content of the barley was about 20%. The sampling error was very high, no doubt owing to uneven ripening of the stand. Toward the end of the period, the sampling error of duplicates decreased greatly, but variations between consecutive samples remained fairly high, as can be seen in the results for August 21 (Fig. 8). *During this eight day period there was only one day during which the moisture content of the grain definitely followed the course of change of the relative humidity.* This was August 20. The weather on that day was distinguished from preceding days only by a slightly higher maximum temperature, 92.5° as against 89.9° the preceding day, and the highest average wind velocity for the period. On the whole, the moisture content either changed contrariwise to the humidity or was independent of it.

Another section of data suitable for studying the relation of relative humidity to moisture content of grain is the data in Table V for August 8–20 inclusive, at Saskatoon. This was a period of clear hot weather with moderate winds. Total recorded rainfall during the 13 days was 0.05 in. in two brief showers on August 9 and 15. A brief summary of the weather for this period is given in Table IX.

TABLE IX

SUMMARY OF WEATHER AT SASKATOON, AUGUST 8 TO AUGUST 20 INCLUSIVE, 1933

Factor	For the 13-day period	
	Range	Mean
Maximum temperature	75°–95°	84.5°
Minimum temperature	39°–62°	55°
Maximum relative humidity	75%–97%	88%
Minimum relative humidity	20%–43%	29%
Bright sunlight	4 hr.–13.9 hr.	11 hrs.
Wind velocity, m.p.h.		
8 a.m.	4–19.6	10.5
2 p.m.	7.1–15.8	10.7
8 p.m.	4.1–12.7	9.6

At the beginning of this period, 6 a.m. August 9, the moisture content of the wheat was 17.2%. At 12 noon August 10, it had definitely dropped to below 14.4%. By noon August 12 it was down to 12%. Thereafter samples were taken only three times daily, namely, at 6 a.m., 2 p.m. and 10 p.m. In order to facilitate discussion of these data, the moistures recorded for 6 a.m., 2 p.m. and 10 p.m. during the whole 12-day period have been retabulated in Table X.

The notes given in Table X under the heading "observations" were taken from a daily record of personal observations of the weather conditions, kept during the period of sampling in 1933. By this means one notes slight sprinkles of rain, too small to be recorded at the meteorological station, but large enough to affect the moisture of a sample of wheat collected soon afterwards.

It can be seen that there was little evidence of an increase in moisture from 2 p.m., the time of highest temperature and lowest humidity, to 10 p.m. when the relative humidity was increasing rapidly, owing to lowering of the

TABLE X

MOISTURES OF WHEAT RECORDED AT 6:00 A.M., 2:00 P.M. AND 10:00 P.M. AUGUST 8 TO 20, 1933, AT SASKATOON

Date	Moisture content at			Observations
	6 a.m. %	2 p.m. %	10 p.m. %	
August 9	17.2	15.2	16.4	Slight rain at 4–5:30 a.m.
10	14.1	13.2	13.0	
11	11.6	12.1	11.8	
12	12.0	no sample	11.6	
13	13.1	11.5	10.8	
14	11.1	10.5	9.9	Slight rain at 10:00 p.m.
15	11.3	10.9	10.6	Slight rain during night
16	11.2	11.0	11.1	
17	11.2	10.8	10.8	
18	10.6	10.3	10.1	
19	10.3	9.9	10.0	
20	10.8	10.4	13.4	Slight rain 6–8 p.m.
Mean—Aug. 11 to Aug. 20	11.3	10.8	11.0 10.7*	

* *Omitting Aug. 20.*

temperature. The high value, 13.4%, for August 20 is fully accounted for by the occurrence of a shower between 6 and 8 p.m. The 6 a.m. samples tended to be, on the average, about 0.6% higher in moisture content than those collected at 10 p.m. Two of the three increases greater than 1% are accounted for by showers occurring between the 10 p.m. and the 6 a.m. samplings.

At Edmonton, samples were collected at 2-hr. intervals during the period 6 a.m. Aug. 28 to 10 p.m. Aug. 31, and again during the period Sept. 11 to Sept. 14 inclusive. The moisture range of samples collected during the first period was 19 to 27.3% and during the second period it was 12 to 17.5%. The range of moisture of duplicate samples and the means, together with the relative humidity, hours of sunshine, and precipitation during these two periods, are shown graphically in Fig. 9.

The samples obtained during the first period, Aug. 28 to Aug. 31, were collected under conditions unfavorable for studying the effect of daily changes in relative humidity. On the first day, there were 13.5 hours of sunshine; the relative humidity decreased to 25 at 4 p.m. and then rose sharply to 85 by 12 p.m. This represented normal good harvest weather. The moisture content was lowest in the samples collected at midnight. The succeeding three days were overcast; no bright sunshine was recorded, and the relative humidity did not fall below 64%. The moisture content decreased during Aug. 29 and 30 to a minimum of about 19% at midday and then increased during the night. At noon Aug. 31, a rain of 0.66 in. fell and the moisture content increased to 26%, which was approximately the value at the beginning

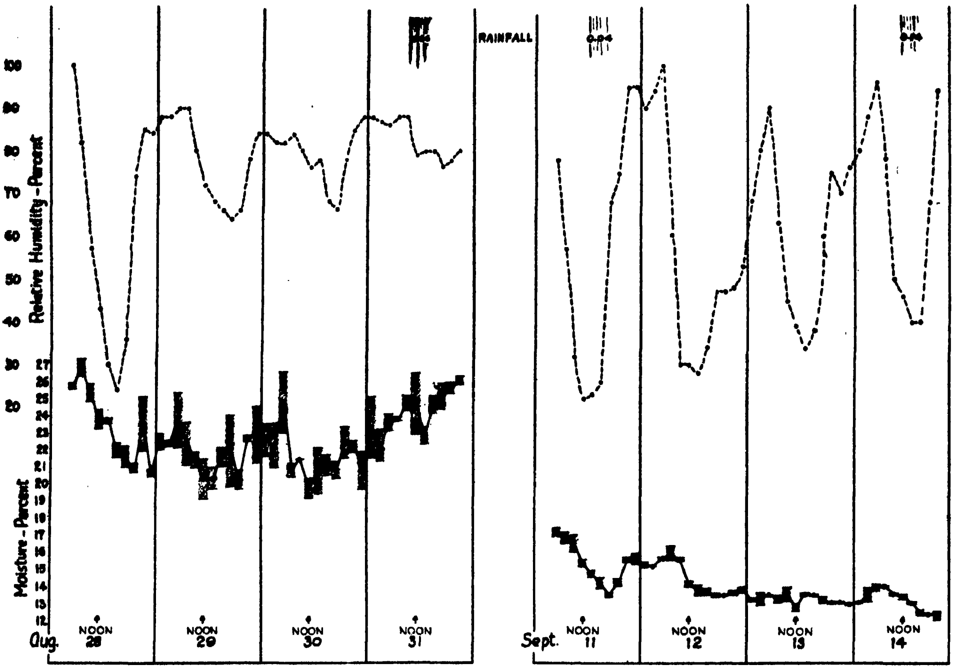

FIG. 9. *Upper and lower limits and average of moisture content of wheat samples collected at 2-hr. intervals for two 4-day periods at Edmonton in 1933.*

of the period. On the whole there seems to have been a rough correspondence between relative humidity and moisture content of the wheat during this 72-hr period.

The second four-day period, Sept. 11 to Sept. 14 inclusive, represented quite characteristic favorable harvesting weather, with bright days, wide ranges of relative humidity and temperature and little rain. The wheat at the beginning had a moisture content of about 17%, which decreased rapidly to 13.5% by 6 p.m. Sept. 11. Rainfall of 0.04 in. occurred between 12 noon and 4 p.m. over a period of three and one-quarter hours. This probably accounts for the increase of moisture content to about 15.5%. Thereafter the moisture content decreased to 13–13.5% and there was no further evidence of increase that could be attributed to changes of relative humidity. As in the results obtained at Winnipeg and Saskatoon, the wheat at Edmonton, having become dried to 13.5% or lower, showed little, if any, fluctuation of moisture content, despite the fact that the relative humidity increased each night to 90% or higher.

These data lead to the conclusion that in clear weather *the increase in relative humidity during the night causes at most only a small increase in moisture content of dry grain.* It cannot be gainsaid that grain just at 14.4% or slightly below this would likely be tough after a night in which the relative humidity rose to 90%, but without the intervention of other factors such as cloudy damp weather and occasional sprinkles of rain, wheat does not remain at 14.4% moisture content during August in Western Canada, but tends rather to decrease to the range 10–12%.

On the basis of the data available, therefore, it seems safe to conclude that wheat, having decreased to below 14.4% in moisture content, stands little chance of becoming tough overnight in normal clear harvest weather, as a result of the increase in relative humidity. Little sprinkles of rain, too small to be recorded, even though of very brief duration, are likely to raise the moisture content of wheat much more rapidly than exposure to the high relative humidity of the night.

Rate of Drying Mature and Immature Wet Standing Grain

Rain during the late harvest season represents such a great hazard both to the farmer and the buyers of wheat that it is desirable to know something of the rates of wetting and drying of standing grain. As it is practically impossible to reproduce the conditions in the laboratory, information on this subject must be obtained in the field. Unfortunately, moderate to heavy rains are frequently followed by days of unsettled weather, during which drying is slow, and therefore it is extremely difficult to compare rates of drying of immature damp grain and mature damp grain. However, there can be selected from Tables III, IV and V several groups of data obtained under conditions that justify their use in comparing the rates of drying of immature and mature damp grain.

TABLE XI

COMPARISON OF RATES OF DRYING OF IMMATURE AND MATURE DAMP STANDING GRAIN

Mature				Immature			
Dates	Moisture range	Moisture decrease	Time, hr.	Dates	Moisture range	Moisture decrease	Time, hr.
Table III							
Aug. 26, 6 a.m. to Aug. 27, 12 a.m.	29.6% to 14.0%	15.6%	30	Aug. 14, 6 p.m. to Aug. 19, noon	28.3% to 13.6%	14.7%	114
Table IV							
Aug. 25, 9 a.m. to Aug. 26, 5 p.m.	29.2% to 14.0%	15.2%	32	Aug. 6, 5 p.m. to Aug. 9, 1 p.m.	28.3% to 13.1%	15.2%	68
Table V							
Sept. 2, 1 p.m. to Sept. 4, 5 p.m.	29.0% to 14.3%	14.7%	52	Aug. 4, 5 p.m. to Aug. 10, 5 a.m.	29.4%* to 14.1%	15.3%	132

* *During this period there was precipitation of 0.21 in. on Aug. 6 and 0.03 in. on Aug. 9.*

In Table XI there are given three sets of observations on the drying of damp mature standing grain and comparable data for immature grain. In all cases the data were selected for periods during which there was good drying weather.

It is impossible to make strict comparisons with immature and mature standing grain, because weather conditions cannot be accurately fixed by description and therefore the best that can be done is to make general observations. It seems reasonably certain that the weather during the early part of August, during which time the grain loses its initial moisture, is at least as favorable from the standpoint of drying as the weather following rains in late August and early September. Therefore, large differences in drying rates would likely have some significance.

It can be seen from the data in Table XI that in all cases cited the mature damp grain dries much more rapidly than the corresponding immature damp grain. The slowest rate with mature grain was sixteen hours faster than the fastest rate with immature grain.

With the data in hand it is impossible to make any rigid comparisons of the rates of wetting and of drying mature grain. The onset of rains often prevented sampling and when the rains started late in the afternoon, a period of eight to twelve hours frequently elapsed before the next sample was taken. All estimates of rate of wetting therefore tend to be low. The most pertinent data obtained in these investigations have been tabulated in Table XII.

TABLE XII

RATE OF WETTING STANDING GRAIN

Table	Place	Date	Hours	Time, hr.	Moisture, %			Precipitation, in.
					Initial	Final	Increase	
III	Winnipeg	Aug. 22 Aug. 23	8 p.m. to 8 a.m.	12	11.1	31.2	20.1	1.22
IV	Saskatoon	Aug. 12	4 p.m. to 8 p.m.	4	12.7	25.9	13.2	0.8
IV	Saskatoon	Aug. 20	8 a.m. to 2 p.m.	6	12.5	27.8	15.3	0.1 (misty)
V	Saskatoon	Aug. 31	10 a.m. to 2 p.m.	4	15.2	23.0	7.8	0.65
V	Saskatoon	Sept. 10	8 a.m. to 8 p.m.	12	15.5	28.1	12.6	1.2
VII	Edmonton	Sept. 14 Sept. 15	8 p.m. to 8 p.m.	24	12.4	26.4	14.0	0.88

Comparing these times with those for drying of mature grain in Table XI, it seems safe to conclude that the rate of wetting is very much faster than the rate of drying. This conclusion scarcely requires demonstration, because in wetting, the water comes in contact with the grain and is absorbed as liquid, whereas in drying the water has to be removed as vapor.

TABLE XIII

TEMPERATURE, HUMIDITY, SUNSHINE, RAINFALL AND WIND VELOCITY RECORD
(University of Manitoba, 1932)

Date	Temperature				Relative humidity				Sunshine, hr.	Rainfall, in.	Wind velocity, m.p.h.
	Max.	Hour	Min.	Hour	Max.	Hour	Min.	Hour			
Aug. 2	78	4 p.m.	67	8 p.m.	89	12 noon	39	4 p.m.	13.1	0.04	12.7
3	80	6 p.m.	65	8 a.m.	61	8 a.m.	30	6 p.m.	12.0	nil	11.6
4	76	12 noon	63	8 p.m.	69	8 p.m.	37	4 p.m.	8.8	0.06	10.3
5	72	2 p.m.	62	8 a.m.	86	8 a.m.	47	2 p.m.	7.0	trace	10.5
6	71	2 p.m.	57	8 a.m.	94	8 a.m.	49	2 p.m.	8.1	0.06	11.8
7	72	2 p.m.	61	8 a.m.	79	8 a.m.	52	12 noon	11.9	0.02	10.4
8	77	12 noon	63	8 a.m.	78	8 a.m.	41	2 p.m.	13.3	nil	7.1
9	82	4 p.m.	65	8 p.m.	58	10 a.m.	36	4 p.m.	14.0	nil	4.4
10	82	12 noon	67	8 a.m.	64	8 a.m.	37	2 p.m.	13.5	nil	5.9
11	82	2 p.m.	63	8 p.m.	67	8 a.m.	35	4 p.m.	12.1	nil	8.5
12	84	2 p.m.	66	8 a.m.	82	8 a.m.	30	4 p.m.	12.4	nil	10.9
13	86	4 p.m.	70	8 a.m.	66	8 a.m.	31	4 p.m.	10.9	nil	13.6
14	88	6 p.m.	78	8 a.m.	59	10 a.m.			8.9	0.03	10.8
15	89	12 noon	74	8 a.m.	61	10 a.m.	32	2 p.m.	12.8	nil	8.3
16	90	2 p.m.	70	8 a.m.	77	8 a.m.	31	4 p.m.	12.0	nil	9.2

TABLE XIV

TEMPERATURE, HUMIDITY, SUNSHINE, RAINFALL AND WIND VELOCITY RECORD
(University of Manitoba, 1933)

Rate	Temperature				Relative humidity				Sunshine, hr.	Rainfall, in.	Wind velocity, ave.
	Max.	Hour	Min.	Hour	Max.	Hour	Min.	Hour			
Aug. 9	87	2 p.m.	77.5	8 p.m.	53	8 p.m.	42	6 p.m.	13.0	0.06	10.7
10	76	4 p.m.	59.5	6 a.m.	89	10 p.m.	38	4 p.m.	12.7	—	11.8
11	79.5	noon	62.7	10 p.m.	74	10 p.m.	37	2 p.m.	12.5	—	15.2
12	70	4 p.m.	54.8	10 p.m.	100	6 a.m.	40	4 p.m.	13.1	—	11.6
13	75.2	4 p.m.	48.5	6 a.m.	100	6 a.m.	39	6 p.m.	13.8	—	9.2
14	85	4 p.m.	46.5	6 a.m.	100	6 a.m.	32	2 p.m.	13.8	—	5.8
15	89	2 p.m.	59	6 a.m.	65	12 p.m.	28	8 p.m.	3.7	0.02	8.7
16	80	2 p.m.	60	12 p.m.	95	6 a.m.	40	6 p.m.	9.6	—	14.3
17	69.5	4 p.m.	50.5	12 p.m.	93	12 p.m.	32	noon	10.3	—	13.9
18	76	4 p.m.	44.5	6 a.m.	100	6 a.m.	28	2 p.m.	12.5	—	5.6
19	89.8	2 p.m.	53	6 a.m.	75	6 a.m.	32	4 p.m.	12.2	—	14.5
20	92.5	4 p.m.	68	6 a.m.	67	6 a.m.	31	6 p.m.	12.7	—	18.6
21	80	4 p.m.	62.5	6 a.m.	89	6 a.m.	53	6 p.m.	1.6	—	12.0
22	80.5	4 p.m.	68	4 a.m.	81	10 a.m.	61	4 p.m.	4.7	1.22	9.8
23	78	6 p.m.	66	8 a.m.	100	8 a.m.	60	6 p.m.	6.3	.15	9.4
24	60.5	6 a.m.	60	8 a.m.	100	8 a.m.	—	—	—	.43	11.2
25	68.5	noon	59	6 p.m.	100	6 p.m.	58	noon	6.6	0.02	9.8
26	69	2 p.m.	47	6 a.m.	94	8 a.m.	51	2 p.m.	10.4	—	8.7
27	67	noon	63	8 a.m.	57	8 a.m.	48	10 a.m.	13.1	—	12.4

TABLE XV

TEMPERATURE, HUMIDITY, SUNSHINE, RAINFALL AND WIND VELOCITY RECORD
(University of Saskatchewan, 1932)

Date	Temperature				Relative humidity				Sunshine, hr.	Rainfall, in.	Wind velocity		
	Max.	Hour	Min.	Hour	Max.	Hour	Min.	Hour			4 a.m.	Noon	8 p.m.
Aug. 4	75	4 p.m.	52	12 p.m.	85	12 p.m.	31	2 p.m.	11.8	—	15.2	13.5	8.2
5	73	3 p.m.	46	5 a.m.	92	7 a.m.	29	1 p.m.	13.1	trace	12.6	10.5	6.7
6	75	6 p.m.	44	5 a.m.	95	12 p.m.	40	5 p.m.	11.5	—	8.6	7.5	2.2
7	83	4 p.m.	50	5 a.m.	96	6 a.m.	22	4 p.m.	14.3	—		4.1	9.2
8	89	6 p.m.	50	8 a.m.	80	8 a.m.	18	6 p.m.	14.2	—	4.8	13.6	15.7
9	92	4 p.m.	56	6 a.m.	72	4 a.m.	19	4 p.m.	14.0	—	11.8	17.0	19.7
10	91	4 p.m.	62	6 a.m.	69	6 a.m.	22	4 p.m.	10.4	—	23.2	15.6	18.7
11	70	11 a.m.	58	7 a.m.	96	12 p.m.	59	9 a.m.	0.5	—	21.8	21.1	13.8
12	78	3 p.m.	53	6 a.m.	100	5 a.m.	57	9 a.m.	7.6	0.04	9.3	3.8	4.6
13	78	4 p.m.	51	5 a.m.	97	3 a.m.	35	3 p.m.	13.4	0.75	8.6	6.8	6.3
14	78	3 p.m.	50	5 a.m.	94	5 a.m.	41	2 p.m.	14.1	—	12.8	9.0	5.2
15	83	5 p.m.	53	6 a.m.	94	6 a.m.	33	5 p.m.	13.3	—	4.8	6.6	4.2
16	77	2 p.m.	57	2 a.m.	91	6 a.m.	41	4 p.m.	11.9	trace	9.2	10.5	2.8
17	78	2 p.m.	45	4 a.m.	95	4 a.m.	27	2 p.m.	13.9	—	7.3	6.3	4.2
18	88	2 p.m.	50	3 a.m.	77	2 a.m.	30	2 p.m.	13.5	—	1.6	1.8	1.5
19	92	2 p.m.	54	5 a.m.	91	12 p.m.	30	2 p.m.	10.5	—	2.5	4.3	1.7
20	63	12 noon	55	12 p.m.	100	12 p.m.	82	8 a.m.	none	—	6.9	8.0	8.6
21	no record				100	5 a.m.			none	0.04	12.5	5.7	3.2
22	66	4 p.m.			97	12 p.m.			none	0.19	2.6	1.0	0.2
23	71	4 p.m.	57	8 a.m.	100	8 a.m.	70	2 p.m.	4.0	0.11	no record		
24	76	6 p.m.	60	7 a.m.	100	8 a.m.	60	6 p.m.	5.5	0.15	2.5	5.1	1.0
25	82	5 p.m.	51	7 a.m.	98	7 a.m.	36	3 p.m.	13.1	0.03	no record		
26	82	4 p.m.	52	6 a.m.	95	7 a.m.	30	4 p.m.	13.3	—	6.0	9.6	9.8
27	85	3 p.m.	51	7 a.m.	91	6 a.m.	25	2 p.m.	13.3	—	11.3	24.5	18.7

TABLE XVI

TEMPERATURE, HUMIDITY, SUNSHINE, RAINFALL AND WIND VELOCITY RECORD
(University of Saskatchewan, 1933)

Date	Temperature				Relative humidity				Sunshine, hr.	Rainfall, in.	Wind velocity		
	Max.	Hour	Min.	Hour	Max.	Hour	Min.	Hour			8 a.m.	2 p.m.	8 p.m.
Aug. 1	70	2 p.m.	34.5	4 a.m.	85	12 p.m.	28	2 p.m.	9.4	—	3.6	5.5	7
2	78	2 p.m.	50.5	2 a.m.	75	12 p.m.	27	2 p.m.	8.4	—	7	13.2	14
3	80.5	2 p.m.	50	4 a.m.	75	12 p.m.	29	2 p.m.	4.0	—	17.5	18.7	19.5
4	93	2 p.m.	60.5	3 a.m.	89	6 p.m.	22	10 a.m.	10.7	—	15	11.6	5.4
5	86.5	2 p.m.	56	1 a.m.	93	8 p.m.	30	12 noon	9.8	—	6.3	13.1	6.2
6	78.5	2 p.m.	53.5	6 a.m.	96	8 p.m.	39	12 noon	8.5	0.21	13.4	10.5	6.1
7	86	4 p.m.	51	2 a.m.	83	12 p.m.	25	12 noon	8.9	—	7.5	8	11.5
8	86	2 p.m.	58	5 a.m.	92	10 p.m.	25	2 p.m.	13.9	—	12.6	10.0	7.5
9	79	12 noon	53	10 p.m.	90	10 p.m.	22	6 a.m.	10.7	0.03	18.7	12.0	6.3
10	83.8	3 p.m.	50	4 a.m.	83	12 p.m.	26	12 noon	10.5	—	10.2	7.3	7.4
11	84	1 p.m.	57	10 p.m.	87	9 p.m.	33	12 noon	12.9	—	14.1	10.0	7.4
12	82.5	1 p.m.	54.5	2 a.m.	89	8 p.m.	39.5	12 noon	8.4	—	8.0	7.1	6.5
13	88.5	12 noon	54.5	2 a.m.	86	8 p.m.	30	12 noon	13.4	—	4.0	7.7	4.3
14	95	2 p.m.	56	2 a.m.	—	—	25	12 p.m.	13.1	—	5.6	15.8	6.8
15	86	4 p.m.	62	8 p.m.	89	11 p.m.	21	1 a.m.	11.8	0.02	10.8	12.5	9.0
16	75	4 p.m.	56	6 a.m.	97	10 p.m.	36	12 noon	12.3	—	19.6	10.7	—
17	77	4 p.m.	39	6 a.m.	90	12 p.m.	30	2 p.m.	11.2	—	4.0	15.0	12.7
18	92	4 p.m.	57.5	6 a.m.	75	12 p.m.	20	12 noon	11.2	—	12.4	12.9	11.6
19	84	4 p.m.	55.5	6 a.m.	93	10 p.m.	35	2 p.m.	4.0	—	6.3	9.2	6.3
20	85	2 p.m.	60	10 p.m.	91	12 p.m.	43	12 noon	10.0	—	10.7	8.5	4.1
21	74	4 p.m.	54.5	6 a.m.	91	12 p.m.	—	—	5.6	0.22	3.5	5.6	5.8
22	78.5	4 p.m.	43.5	6 a.m.	90	8 p.m.	23	12 noon	6.6	—	10.5	6.5	4.5
23	75	2 p.m.	59	10 p.m.	80	1 a.m.	41	12 noon	0.5	—	19.1	13.2	8.3
24	62.5	4 p.m.	52	10 p.m.	96	8 p.m.	61	2 p.m.	0.0	trace	8.8	8.3	4.5
25	70	2 p.m.	36.5	4 a.m.	84	4 p.m.	30	12 noon	13.7	0.02	8.2	7.0	7.7
26	80	2 p.m.	47	11 p.m.	96	6 p.m.	23	10 a.m.	12.7	—	9.8	8.3	0.5
27	75.5	1 p.m.	37	4 a.m.	75	11 p.m.	21	10 a.m.	13.6	—	2.7	8.3	10.2
28	83.4	3 p.m.	47	1 a.m.	80	12 p.m.	28	12 noon	13.1	—	16.6	13.5	11.0
29	85.4	2 p.m.	55.5	4 a.m.	92	10 p.m.	37	2 p.m.	13.2	—	9.1	5.6	8.1
30	88	3 p.m.	52.5	6 a.m.	95	12 p.m.	25	12 noon	10.0	—	6.3	10.5	9.2
31	69.5	1 a.m.	54	6 a.m.	100	2–12 p.m.	96	6 a.m.	0.0	0.01	6.1	8.0	12.0

TABLE XVI—*Concluded*

Temperature, humidity sunshine, rainfall and wind velocity record
(University of Saskatchewan, 1933)

Date	Temperature				Relative humidity				Sunshine, hr.	Rainfall, in.	Wind velocity		
	Max.	Hour	Min.	Hour	Max.	Hour	Min.	Hour			8 a.m.	2 p.m.	8 p.m.
Sept. 1	57	12 noon	46.5	11 p.m.	100	noon	83	12 p.m.	0.0	0.65	23.0	30.0	25.2
2	59	2 p.m.	44.5	10 p.m.	95	8 p.m.	48	12 noon	4.5	0.55	20.0	10.1	6.7
3	62	2 p.m.	37	4 a.m.	85	10 p.m.	29	10 a.m.	12.6	—	3.6	14.2	21.5
4	82	2 p.m.	46	2 a.m.	75	12 p.m.	22	8 a.m.	10.8	—	3.6	14.2	21.5
5	63	3 p.m.	43.5	11 p.m.	95	12 p.m.	41	2 p.m.	8.5	—	12.2	12.7	12.1
6	48	4 p.m.	39.5	6 a.m.	100	8 p.m.	92	8 a.m.	0.0	0.21	11.6	8.6	8.6
7	52	2 p.m.	39.5	4 a.m.	97	2 a.m.	85	10 a.m.	0.0	0.02	7.2	5.6	5.5
8	72.5	2 p.m.	48	4 a.m.	90	9 p.m.	33	12 noon	9.9	—	11.8	10.7	7.1
9	80	2 p.m.	47	4 a.m.	89	8 p.m.	32	10 a.m.	12.7	—	2.3	6.6	8.8
10	66	6 a.m.	52.5	10 p.m.	98	8 p.m.	55	4 a.m.	0.0	—	10.5	8.6	5.6
11	67	6 p.m.	50	2 a.m.	97	12 p.m.	75	6 p.m.	2.5	1.2	3.2	5.8	2.1
12	63	2 p.m.	51	10 p.m.	100	10 p.m.	71	10 a.m.	0.3	0.002	4.1	5.3	7.5
13	61	4 p.m.	50	4 a.m.	100	2 a.m.	75	2 p.m.	3.1	—	13.5	16.7	24.7
14	65.5	4 p.m.	48	6 a.m.	92	2 a.m.	71	2 p.m.	7.2	—	31.7	27.7	24.1
15	60.5	1 a.m.	53.5	10 p.m.	100	2–12 p.m.	83	1 a.m.	0.0	—	19.8	13.5	7.1
16	59	4 p.m.	52.5	11 p.m.	96	8 p.m.	68	2 p.m.	10.3	0.1	15.6	9.6	6.5
17	69.5	2 p.m.	40	4 a.m.	86	8 p.m.	—	—	10.3	—	4.6	8.5	10.1
18	62.5	2 p.m.	48.5	6 a.m.	76	8 a.m.	67	6 p.m.	4.6	—	13.0	18.5	14.1

TABLE XVII

TEMPERATURE, HUMIDITY, SUNSHINE, RAINFALL AND WIND VELOCITY RECORD
(University of Alberta, 1932)

Date	Temperature				Relative humidity				Sunshine, hr.	Rainfall, in.	Average wind velocity, m.p.h.
	Max.	Hour	Min.	Hour	Max.	Hour	Min.	Hour			
Aug. 23	83	4 p.m.	46	8 a.m.	64	8 a.m.	22	4 p.m.	12.3	—	2.4
24	85	2–4 p.m.	58	8 a.m.	50	8 p.m.	20	4 p.m.	12.2	0.14	2.4
25	88	2 p.m.	59	8 a.m.	50	8 a.m.	20	2 p.m.	13.4	—	4.5
26	85	2 p.m.	66	8 a.m.	62	8 p.m.	39	2 p.m.	13.1	—	7.3
27	87	4 p.m.	62	8 a.m.	65	8 a.m.	32	4 p.m.	13.1	—	6.8
28	63	12 noon	55	8 p.m.	73	8 a.m.	47	12 noon	4.5	—	6.6
29	54	4 p.m.	49	8 p.m.	64	8 a.m.	31	4 p.m.	0.3	—	5.9
30	59	2 p.m.	46	8 a.m.	79	8 a.m.	21	4 p.m.	8.0	—	8.5
31	64	6 p.m.	34	8 a.m.	85	8 a.m.	49	4 p.m.	10.6	0.01	6.6
Sept. 1	61	4 p.m.	43	8 a.m.	100	6 p.m.	62	4 p.m.	6 0	0.02 (6 p.m.)	8.5
2	58	4 p.m.	45	8 a.m.	87	6 p.m.	55	12 noon	6.6	0.01 (4 p.m.)	11.4
3	65	4 p.m.	40	8 a.m.	71	8 p.m.	25	2 p.m.	8.4	—	4.7
4	72	6 p.m.	47	8 a.m.	75	8 a.m.	32	12 noon	8.8	—	4.6
5	79	4 p.m.	42	8 a.m.	79	8 p.m.	41	4 p.m.	11.6	—	2.8
6	83	2 p.m.	49	8 a.m.	70	8 a.m.	26	4 p.m.	9.6	—	4.3
7	80	2 p.m.	65	8 p.m.	84	8 p.m.	42	2 p.m.	11.5	—	5.7
8	61	8 a.m.	55	2 p.m.		rain				0.13 (4 p.m.)	16.2
9	73	2 p.m.	51	8 p.m.	42	4 p.m.	88	8 p.m.	11.4	—	6.2
10	79	12 noon	64	8 p.m.	65	8 p.m.	28	12 noon	11.2	—	11.6
11	64	8 a.m.	44	8 p.m.	87	8 p.m.	47	12 noon	2.3	Trace	7.8
12	61	2 p.m.	53	8 p.m.	81	8 p.m.	44	12 noon	6.8	—	4.4
13	78	4 p.m.	48	8 a.m.	67	8 a.m.	20	12 noon	8.6	—	8.6
14	61	2 p.m.	53	8 p.m.	81	8 p.m.	41	12 noon	10.7	—	17.0

TABLE XVIII

TEMPERATURE, HUMIDITY, SUNSHINE, RAINFALL AND WIND VELOCITY RECORD
(University of Alberta, 1933)

Date	Temperature				Relative humidity				Sunshine, hr.	Rainfall, in.	Wind vel., ave.
	Max.	Hour	Min.	Hour	Max.	Hour	Min.	Hour			
Aug. 19	76	2 p.m.	59	10 p.m.	96	10 p.m.	58	2 p.m.	7.9	0.02	9.0
20	73	6 p.m.	50	6 a.m.	90	10 p.m.	36	2 p.m.	6.1	—	5.2
21	84	2 p.m.	45	6 a.m.	90	10 p.m.	27	noon	13.9	—	5.0
22	92	2 p.m.	46	6 a.m.	100	6 a.m.	32	2 p.m.	13.3	0.27	5.0
23	62	noon	52	10 p.m.	100	6 a.m.	60	4 p.m.	0.9	0.17	6.7
24	72	2 p.m.	44	6 a.m.	100	6 a.m.	38	noon	13.3	—	4.0
25	80	2 p.m.	36	6 a.m.	100	6 a.m.	30	2 p.m.	13.9	—	4.0
26	76	2 p.m.	34	6 a.m.	100	6 a.m.	30	4 p.m.	13.5	—	4.4
27	82	4 p.m.	43	6 a.m.	93	6 a.m.	24	2 p.m.	13.5	—	8.6
28	90	4 p.m.	46	6 a.m.	100	6 a.m.	24	4 p.m.	13.5	—	4.7
29	61	6 p.m.	46	6 a.m.	100	6 a.m.	64	6 p.m.	—	—	5.4
30	58	4 p.m.	50	10 p.m.	85	10 p.m.	66	6 p.m.	—	—	2.6
31	54	10 a.m.	50	2-10 p.m.	88	10 a.m.	76	6 p.m.	—	0.66	8.7
Sept. 1	62	2 p.m.	50	8 a.m.	96	8 a.m.	58	6 p.m.	4.0	—	12.9
2	68	4 p.m.	38	6 a.m.	100	6 a.m.	33	4 p.m.	12.0	—	6.6
3	69	6 p.m.	39	6 a.m.	100	6 a.m.	39	4 p.m.	8.4	—	8.8
4	60	2 p.m.	46	6 a.m.	100	6 a.m.	50	2 p.m.	7.8	—	9.2
5	41	4 p.m.	33	6 a.m.	100	6-12 a.m.	88	6 p.m.	—	0.07	5.0
6	44	2 p.m.	36	6 a.m.	100	6-10 p.m.	89	6 a.m.	—	0.09	2.7
7	62	4 p.m.	42	6 a.m.	98	8 a.m.	55	4 p.m.	3.1	—	3.0
8	82	2 p.m.	40	6 a.m.	100	6 a.m.	30	2 p.m.	9.5	—	5.7
9	89	4 p.m.	43	6 a.m.	100	6 a.m.	23	2 p.m.	11.9	—	3.4
10	67	4 p.m.	53	6 a.m.	90	10 p.m.	57	noon	1.8	0.05	3.7
11	70	2 p.m.	38	10 p.m.	95	10 p.m.	22	noon	10.0	0.04	4.0
12	71	2 p.m.	34	6 a.m.	100	6 a.m.	28	2 p.m.	7.4	—	9.4
13	73	2 p.m.	40	6 a.m.	90	6 a.m.	34	2 p.m.	7.1	—	5.4
14	75	2 p.m.	47	6 a.m.	98	10 p.m.	40	2 p.m.	5.8	0.14	5.6
15	53	10 a.m.	47	10 p.m.	98	6 p.m.	94	2 p.m.	—	0.64	8.0
16	57	2 p.m.	41	10 p.m.	93	6 a.m.	46	2 p.m.	8.0	0.21	13.7

Conclusion

In applying the information obtained in this study to the problem of avoiding tough grain in straight combining, the following general observations may be made.

Under the most favorable weather conditions, four to seven days must elapse from the time grain is fit to cut with the binder until it can be harvested by straight combining to obtain straight grade grain. Mature grain usually dries to 11–13% moisture content in areas having favorable harvest weather, and thereafter there is practically no danger of the moisture rising above 14.4% during the night, except through the occurrence of rains or mists or protracted periods of damp cloudy weather. The "toughness" that normally occurs at night during clear weather has to do with the decrease in brittleness of the straw, rather than with increase of moisture of the wheat. Rains, even though of brief duration, cause a very rapid increase in moisture content of dry wheat. Increases of from 12.7% to 26% in no more than four hours have been observed. The subsequent drying is much slower than the wetting. It might be expected that at least 24 hours of the most favorable weather would be required to reverse a change such as that noted above. This is likely to lead to an error in judgment on the part of combine operators, which will result in tough grain being threshed, because the straw becomes brittle and fit for threshing before the moisture of the grain has decreased to below the "tough" limit.

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QUALITY AND KEEPING PROPERTIES OF FLOUR FROM WHEAT GROWN ON THE BLACK AND GRAY SOILS OF ALBERTA[1]

By O. S. Aamodt[2] and A. G. McCalla[3]

Abstract

Weight per bushel and milling yields of hard red spring wheats grown on the black soil at Edmonton were the same as for the same varieties grown on the gray soil at Fallis. Grade, protein content and baking quality of Edmonton grown samples were superior. The flour from most of the Edmonton grown samples retained its quality for at least two years after milling, but flour from most of the Fallis grown samples had deteriorated so much during 10 months storage that it was unfit for breadmaking. Flour from Reward showed less deterioration than that from any other standard variety grown at Fallis. It also had the best original baking quality, and is the only one of the recommended varieties considered satisfactory for the gray soil area.

Introduction

The gray wooded soil belt which occupies a large proportion of western and northern Alberta is sparsely settled at the present time, but, because of favorable moisture conditions, the trend of settlement is toward this area. Much of the soil is relatively low in fertility and requires special management if satisfactory results are to be obtained (9). Only recently have experimental plots been established on these soils, the early work being carried out by the Department of Soils of the University of Alberta. This work has been concerned with the fertility and management of the soil rather than with the quality of the products.

During the past few years the Department of Field Crops of the University of Alberta has located experimental plots at Fallis, a point 50 miles west of Edmonton. The nature and composition of the gray soil in this area, and of the black loam soil typical of the Edmonton district are described by Wyatt, Newton and Mather (10). The soil at Fallis is a wooded podsolic loam which forms the main class of well drained soil found in wooded areas. The quality of wheat grown at Fallis is probably fairly typical of that for the wheat grown in the gray soil belt as a whole.

This paper deals with the results obtained in a study of the quality of wheat grown at Edmonton and Fallis in 1932 and 1933.

Materials and Methods

The material used in this study was grown in replicated rod row plots at Edmonton and Fallis, in connection with the routine testing of hard red spring wheat standard varieties and hybrids. The quality of each of the named varieties Garnet, Huron, Marquis, Red Bobs 222 and Reward has

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been discussed by Malloch, Geddes and Larmour (6), who concluded that Reward, Marquis and Red Bobs 222 were satisfactory for export and domestic milling. Garnet and Huron were both considered inferior to Marquis in baking quality. Caesium is a Russian wheat obtained from Dr. Talanov of the West Siberian Experimental Station in 1928. It is usually inferior to Marquis in protein content and baking quality. The hybrids I-28-60 and I-28-65 are selections made from a Marquillo × Marquis-Kanred cross introduced from the Minnesota Experimental Station. Both are satisfactory in protein content and in milling and baking quality.

All samples were graded at the Edmonton office of the Western Grain Inspection Division. Average grades were calculated by assigning numerical values of from 0 to 4 to Grades 1 Hard, 1, 2, 3 and 4 Northern respectively. This method has been used by Larmour, Geddes and Cameron (5).

Nitrogen was determined by the Kjeldahl method with mercuric oxide as catalyst. Protein values are reported as total nitrogen × 5.7, and corrected to a 13.5% moisture basis.

Milling tests were carried out on an experimental mill following the flow sheet presented by Geddes (3). Straight flour was produced in all tests.

The baking results reported were obtained using the bromate formula with 1 mg. of potassium bromate per loaf (2). The simple formula was also used in 1932, and the malt-phosphate-bromate in 1933, but the results obtained with these formulas led to essentially the same conclusions as those obtained with bromate, so they are not included in the tables.

Results

Miscellaneous and Milling Tests

The results of miscellaneous and milling tests are presented in Table I.

Edmonton grown samples averaged about 1.5 grades higher than Fallis grown in both years, although the average for 1933 samples from each place was about one grade lower than for 1932 samples. There was a marked difference in the effect of soil on the grade of different varieties. Reward and Garnet showed the least tendency for grade reduction when grown on the gray soil, but since by regulation of the Grain Inspection Division, 2 Northern was the highest grade which could be granted Garnet, the results for this variety do not necessarily indicate that there were no differences in the external characteristics of the samples.

Individual differences in weight per bushel cannot be regarded as significant. The maximum difference between Edmonton- and Fallis-grown samples of the same variety was 2.5 lb.; but the differences in some varieties favored one, and in others the other, sample.

The protein content of Fallis grown samples was lower than that of Edmonton grown for each variety, although there were varietal differences in the degree of variation. The average protein content of Edmonton grown samples was 3.4% higher in 1932, and 4.0% higher in 1933, than that of

TABLE I

Comparison of quality of wheat grown at Edmonton and Fallis

Variety	Grade*		Wt. per bushel, lb.		Protein, %		Yield of straight flour, %		Loaf volume, cc.		Partial baking score	
	Edmonton	Fallis	Edmonton	Fallis	Edmonton	Fallis	Edmonton	Fallis	Edmonton	Fallis	Edmonton	Fallis
1932												
Caesium	2°	3°	67	65.5	14.0	11.0	69.1	70.6	672	622	57	53
Garnet	2°	2°	63.5	65	14.1	11.8	70.1	67.3	648	618	54	52
Huron	1°	2°	64	63.5	14.6	11.1	69.2	70.5	670	586	54	54
Marquis	1 Hard	2°	64.5	65	15.2	11.0	70.0	73.1	721	600	52	51
Red Bobs	1 Hard	3°	64	64	14.2	10.3	70.6	71.8	632	588	57	52
Reward	1 Hard	1 Hard	64.5	67	16.5	13.6	69.1	72.3	708	662	55	57
Av. of 18	0.9	2.3	64.3	64.3	14.9	11.5	69.0	69.6	703	619	54	53
1933												
Caesium	2°	4°	67	66	16.0	11.1	71.4	70.5	730	603	44	36
Garnet	2°	2°	65.5	65.5	14.1	11.7	70.8	69.8	672	588	53	43
Huron	2°	4°	65	65	15.1	10.4	72.2	69.0	663	558	58	36
Marquis	1°	4°	66	65	15.3	10.7	69.8	69.5	660	548	56	41
Red Bobs	1°	3°	66	64.5	14.7	10.6	71.4	71.6	694	632	53	45
Reward	1°	2°	66	67	16.8	12.6	68.2	68.1	795	627	59	51
Ave. of 11	1.9	3.4	65.5	65.2	15.2	11.2	70.2	69.6	696	603	53	42

*1° = No. 1 Northern; 2 = No. 2 Northern, etc.

the Fallis grown. The results obtained in this study represent fairly well the relative differences in protein content of samples grown in the black and gray soil areas.

Individual differences in milling results, like those in weight per bushel, cannot be considered significant. The average flour yields from samples grown at both places in both years were practically the same.

Baking Tests Made One Month after Milling

The first baking tests were made approximately one month after the wheat was milled. The loaf volume and the partial baking score (1) results are presented in Table I.

For both years the loaf volume of each individual Edmonton grown sample was larger than that of the comparable Fallis grown sample. The average volume of loaves from Edmonton-grown material was about 90 cc. greater than the average of those from the Fallis grown. The results for the two years are essentially the same, and show that the differences are probably to be expected in any comparison of material from the two places.

The partial baking scores of the two groups of samples collected in 1932 were similar, but those for the 1933 samples show a marked superiority of Edmonton grown material. There were decided varietal differences in 1933 among Fallis grown samples, the distinct superiority of Reward and inferiority of Caesium and Huron being outstanding. Reward was the only variety which could be considered of satisfactory quality when grown at Fallis, and it was distinctly inferior to the Edmonton grown sample of the same variety.

Correlation studies on these and similar samples have been made by Aamodt and Torrie (1). These studies showed that loaf volume and protein content were more closely correlated for Fallis grown than for Edmonton grown material. This was to be expected because the general level of protein content of Fallis grown samples was low and in the range where differences are most important in their effect on loaf volume. The same relation was found for the correlations between partial baking score and protein, but partial correlations showed that protein content was more closely related to loaf volume than to partial baking score.

Baking Tests Made Ten Months after Milling

Fifteen of the original 18 pairs of samples grown in 1932 were re-baked approximately ten months after milling. The flour had been stored in cans with fairly close fitting tops, so that the moisture loss during storage was not over 1%, and at the time of the second baking the moisture content of the samples was between 11 and 12%. Unfortunately most of the flour from Reward and Marquis samples had been used for other purposes, so these varieties could not be included in the second test. The results obtained, together with those for the original baking, are presented in Table II.

These results show that, while the flour from the Edmonton samples made just as good bread after ten months' storage as after one month, that from some of the Fallis samples had deteriorated badly. There was considerable

TABLE II

BAKING RESULTS OF FLOUR SAMPLES FROM EDMONTON AND FALLIS WHEAT

Variety	Origin	Loaf volume, c.c			Partial baking score		
		Stored 1 mo.	Stored 10 mo.*	Change after 10 mo.* storage	Stored 1 mo.	Stored 10 mo.*	Change after 10 mo.* storage
1932							
Caesium	Edmonton	672	729	57	57	54	− 3
	Fallis	622	459	−163	52	37	−15
Garnet	Edmonton	648	592	− 56	54	54	0
	Fallis	618	392	−226	52	32	−20
Huron	Edmonton	670	637	− 33	54	54	0
	Fallis	586	572	− 14	54	48	− 6
I-28-60	Edmonton	770	782	12	52	56	4
	Fallis	650	364	−286	57	31	−26
I-28-65	Edmonton	695	728	33	58	57	− 1
	Fallis	638	590	− 48	55	54	− 1
Red Bobs	Edmonton	632	666	34	57	55	− 2
	Fallis	588	428	−160	52	39	−13
Average of 15	Edmonton	694	700	6	54	54	0
	Fallis	617	503	−114	53	41	−12
1933							
Garnet	Edmonton	672	456	−216	53	46	− 7
	Fallis	588	414	−174	43	33	−10
Marquis	Edmonton	660	610	− 50	56	56	0
	Fallis	548	473	− 75	41	45	4
Red Bobs 222	Edmonton	694	624	− 68	53	58	5
	Fallis	632	440	−192	45	33	−12
Reward	Edmonton	795	644	−151	59	58	− 1
	Fallis	627	586	− 41	51	51	0

**1933 samples stored 12 months.*

variation in the varietal reaction with respect to the deterioration. The two selections I-28-60 and I-28-65 represent the extremes, the former showing a decrease of 286 cc. in loaf volume, and a reduction of 26 in partial baking score, and the latter a decrease of 48 cc. in volume, and a reduction of 1 in baking score. One hybrid showed an increase of 18 cc. in loaf volume but a slight decrease in baking score.

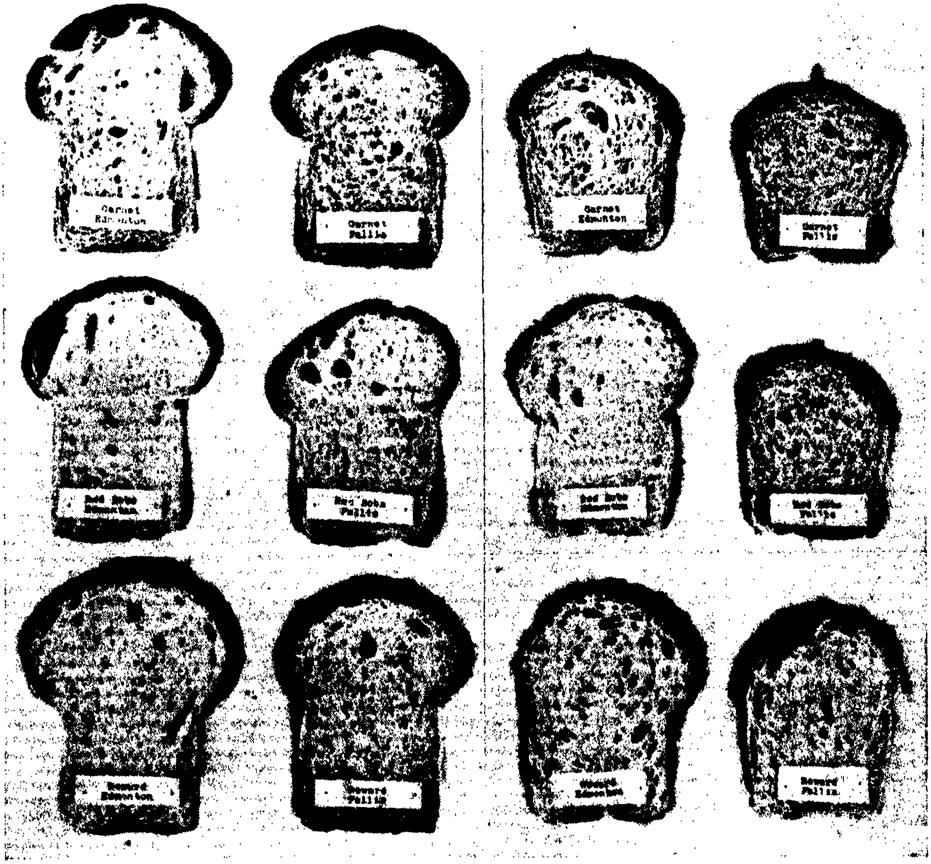
The loaves baked from badly deteriorated flour were poor in all respects. They were dull in crust color, rough in form, coarse and soggy in texture, and dark in crumb color. These characteristics were most marked in the poorest loaves, and the extent to which they appeared was roughly proportional to the decrease in loaf volume.

Flour from some of the varieties was baked 15 months after milling, and the relations obtained after ten months' storage were still found to hold.

Although there was insufficient flour from Reward and Marquis samples to bake a second time, there was sufficient to use in gluten tests. Preliminary studies on deteriorated and normal samples of flour showed that the gluten of deteriorated flour was hard to wash out, and when obtained was coarse, spongy, and dark in color, while that from non-deteriorated flour was easy to wash out and was coherent, elastic and creamy. The Edmonton- and Fallis-grown Reward and the Edmonton grown Marquis yielded normal gluten, but the Fallis grown Marquis appeared to be badly deteriorated.

The 1933 samples were re-baked twelve months after milling. In general the results (Table II) substantiated those obtained with the 1932 samples, but several differences must be noted.

There were decreases in the volumes of the loaves baked from both Edmonton- and Fallis-grown samples, and in the case of Garnet and Reward,



Baked after 1 month's storage. *Baked after 12 months' storage.*

FIG. 1. *The effect of storage on the baking quality of flour, 1933 samples.*

these decreases were greater for Edmonton- than for Fallis-grown. The loaf volumes of Edmonton grown Marquis, Red Bobs 222 and Reward, and Fallis grown Reward, however, were still large enough to be considered satisfactory, and the partial baking scores indicate that real deterioration of quality had not taken place. Fallis grown Marquis was lower in loaf volume than is satisfactory for this type of wheat, and the apparent improvement in partial baking score was due to an abnormally high absorption of water. When allowance was made for this the score was lower than for the first baking. Fallis grown Garnet and Red Bobs were definitely deteriorated, as indicated by marked decreases in both loaf volume and partial baking score, (Fig. 1).

Taking into consideration the lower average level of original quality in the Fallis samples, these results substantiate those obtained for the 1932 samples. Fallis-grown Reward deteriorated little during storage, Marquis deteriorated somewhat more, while Garnet and Red Bobs 222 deteriorated markedly. The deterioration of Edmonton grown Garnet has not been observed before, but emphasizes the necessity of studying this problem further. It was the only standard variety showing this effect although one of the selections gave similar results.

Discussion

The significance of the general low quality of the wheat produced on the gray soil at Fallis in 1933 can hardly be over-emphasized. If the quality of the wheat grown in other parts of the gray soil area is in general as low as in these samples, it is certain to militate against the maintenance of high quality in western Canadian wheat. The annual protein surveys (4) carried out by the Grain Research Laboratory of the Board of Grain Commissioners at Winnipeg show definitely that in many parts of the gray soil area the protein level of the wheat grown is lower by as much as 2 or 3% than that of the Fallis grown samples. Few of the samples obtained from the gray soil belt were much higher in protein than those included in the present study.

It has been shown further that, although yields from the gray soil may be materially increased by the use of fertilizers and green manures (8, 9), protein content of the grain produced is usually not increased, but is actually reduced in many cases (7). While the growing of legumes and the use of fertilizers appear necessary to the proper management of the gray soil, so far there has been no indication that the quality of wheat is materially improved by such management.

Of even greater importance, however, is the fact that the flour produced from many of the standard varieties of wheat grown at Fallis, will not keep when stored under the same conditions as flour milled from the same varieties grown on black soil, although the latter have kept satisfactorily for over two years. Whether the flour from wheat grown in other districts in the gray soil area deteriorates during storage has not been determined, but this point is under investigation at present. The effect of fertilizers on the keeping quality of flour has not been investigated yet, but fundamental studies on the nitrogen and mineral nutrition of wheat grown on both soils are being carried out.

It is important to recognize that the flour from some varieties is apparently not subject to deterioration even after storage for 15 months after milling. At the present time the varieties Garnet, Red Bobs 222, Reward and Marquis are the most commonly grown in the area under discussion. The results of this study indicate that Reward is the most satisfactory in original quality, this quality being high enough to justify the production of the variety in the gray soil area. Fallis grown Reward also appears to be less deleteriously affected by storage than the other standard varieties, and is the only one of the varieties commonly recommended for central and northern Alberta which should be grown on the wooded soils.

Little commercially milled flour is likely to be stored for as long as a year, and commercial storage practices are probably less favorable to the processes of deterioration than those used in the laboratory. The question of deterioration may, however, assume more than academic importance if the volume of wheat from the area under discussion is increased by continued expansion of development in this area.

The results so far reported have all pertained to the study of stored flour. Much of the grain is stored as such for longer periods than it is stored as flour, and this fact has led to a study of the effect of storage on wheat as well as flour. Preliminary results indicate that wheat stored for 15 months after the first milling exhibits little if any of the signs of deterioration exhibited by the flour.

Studies on the chemical changes involved in the deterioration, and on the physiological development of wheat grown on the two soils are being carried out by the junior author and his associates. The results of these studies will be published later.

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SUPERIORITY OF SILVER NITRATE OVER MERCURIC CHLORIDE FOR SURFACE STERILIZATION IN THE ISOLATION OF *OPHIOBOLUS GRAMINIS* SACC.[1]

By F. R. Davies[2]

Abstract

It has been difficult to isolate *Ophiobolus graminis* from plant parts previously surface sterilized by the commonly used mercuric chloride method, although *Helminthosporium sativum* and *Fusarium* spp. are readily isolated following its use. When a silver nitrate method of surface sterilization was substituted, *O. graminis* was isolated with considerably more success than had previously been obtained. These results can be explained by the difference in reaction of the above-mentioned fungi to these chemicals. When similar concentrations of these chemicals were added to nutrient agar, silver nitrate proved less toxic to *O. graminis* than mercuric chloride, whereas the reverse was true for *H. sativum*.

Introduction

In studies on the parasitism of cereal foot- and root-rotting fungi, difficulty was encountered in isolating *Ophiobolus graminis* Sacc. from plant tissue known to be infected. It occurred to the writer that this might be due to mercuric chloride, the surface sterilizing agent used, especially since it is known that certain chemicals have a differential toxicity to different species of fungi.

Mercuric chloride has been found quite satisfactory in the isolation of *Helminthosporium sativum* P. K. and B. and *Fusarium* spp., but when used on material containing *Ophiobolus graminis* and other organisms, the latter usually overgrew the relatively slow growing *O. graminis*.

Apparently other workers have encountered similar difficulty. Broadfoot (2) reports inability to isolate *Ophiobolus graminis* from naturally infected material from the field; he found that other organisms, such as *Helminthosporium sativum* and *Fusarium* spp., grew to the exclusion of *O. graminis*, even though the latter had caused most of the damage. Similarly, Russell (9) reports his invariable failure to isolate *O. graminis* from diseased tissue.

Because of the difficulties mentioned above, it was the purpose of this investigation to find a suitable method of isolating *Ophiobolus graminis* from diseased plant tissues and to explain the unsuitability of mercuric chloride for surface sterilization.

Relative Values of Different Methods of Surface Sterilization in the Isolation of *Ophiobolus graminis* from Plant Tissues

Methods

The material used for the preliminary tests consisted of young wheat stems from plants with pronounced "take-all" symptoms, growing in soil that had been infested with *Ophiobolus graminis*. For the subsequent comparison of mercuric chloride and silver nitrate the material consisted of wheat stubble from plants naturally infected in the field.

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[2] *Formerly graduate assistant in Plant Pathology, University of Alberta, now graduate student at the University of Minnesota, St. Paul, Minnesota, U.S.A.*

Several methods which had previously been used by others for the surface sterilization of seeds were tried. These involved the use of such chemicals as silver nitrate, calcium hypochlorite, and hydrogen peroxide. In addition, a method of washing in sterile water, devised by Simmonds (10), was tried. The results are summarized in Table I. The general method of procedure was as follows. The tissues were cut up into pieces approximately 5 mm. long; these were immersed in the various solutions for varying lengths of time, and then washed in alcohol, sterile sodium chloride, or sterile water, as indicated in Table I; the pieces were then placed on potato-dextrose agar in petri dishes and incubated at room temperature.

TABLE I

THE EFFECT OF VARIOUS CHEMICALS, USED FOR SURFACE STERILIZATION, ON THE SUBSEQUENT ISOLATION OF *Ophiobolus graminis* FROM INFECTED WHEAT PLANTS

Surface sterilizing agent	Concentration	Time of immersion, min.	Rinsing agent	Isolations*
Mercuric chloride	1 : 1,000	2	70% ethyl alcohol	1
	1 : 2,000	2	70% ethyl alcohol	0
	1 : 1,000	2	Sterile water	0
Silver nitrate	1 : 100	2	Sterile sodium chloride and sterile water	4
	1 : 100	5	Sterile sodium chloride and sterile water	3
Calcium hypochlorite	1 : 14	2	Sterile water	0
	1 : 14	5	Sterile water	0
Hydrogen peroxide	3 : 100	2	Sterile water	0
	3 : 100	5	Sterile water	0
None	—	5	Washing in sterile water (Simmonds' method)	0

**Indicates the number of isolations from 12 pieces of infected stems plated.*

Results

Ophiobolus graminis was isolated only when silver nitrate and mercuric chloride were used for surface sterilization (Table I). To ascertain which was preferable, a more extensive test was made with ten samples of wheat

TABLE II

THE EFFECT OF MERCURIC CHLORIDE AND SILVER NITRATE, USED FOR SURFACE STERILIZATION, ON THE SUBSEQUENT ISOLATION OF *Ophiobolus graminis* FROM INFECTED WHEAT PLANTS

Specimen No.	Number of isolations*		Specimen No.	Number of isolations*	
	Mercuric chloride	Silver nitrate		Mercuric chloride	Silver nitrate
2	1	1	141	0	0
23	0	1	191	0	5
73	0	4	201	1	6
88	0	0	247	0	2
126	0	1	292	1	8
			Total	3	28

**Indicates the number of isolations from 12 pieces of infected stem from each specimen.*

stubble that showed typical "take-all" symptoms when collected in Alberta during 1930 and 1931. Twenty-four pieces of diseased stems were cut from each sample, 12 were treated with mercuric chloride and 12 with silver nitrate. Results are given in Table II.

A summary of the results given in Table II shows that *Ophiobolus graminis* was isolated from only three out of the ten samples treated with mercuric chloride, that is from only 3 of the 120 pieces of stubble. On the other hand, it was isolated from 8 of the 10 samples or from 28 of the 120 pieces of stubble treated with silver nitrate.

A third series of isolations made in a similar manner resulted in seven isolations after the use of silver nitrate and only one after using mercuric chloride. Table III summarizes the results obtained from the three series.

TABLE III

SUMMARY OF THE RESULTS OF THREE SERIES OF TESTS ON THE EFFECT OF MERCURIC CHLORIDE AND SILVER NITRATE, USED FOR SURFACE STERILIZATION, ON THE SUBSEQUENT ISOLATION OF *Ophiobolus graminis* FROM INFECTED WHEAT PLANTS

Series	Silver nitrate method		Mercuric chloride method	
	Number of isolations*	Isolation, %	Number of isolations*	Isolation, %
1	7/24	29.3	1/36	2.8
2	28/120	23.3	3/120	2.5
3	7/75	9.3	1/75	1.3
Total	42/219	19.2	5/231	2.2

**Numerator of each fraction indicates the number of pieces of infected stems yielding Ophiobolus graminis, while the denominator indicates the number plated.*

The results given in Table III show a large difference in favor of the silver nitrate method, and from the consistency of the results it seems quite safe to recommend silver nitrate in preference to mercuric chloride as a surface sterilizing agent for the isolation of *Ophiobolus graminis* from diseased wheat stems.* By this method it has been found possible to isolate *O. graminis* from wheat stems several years old. It has also been used in the isolation of this fungus from the dead stems of other grasses and also from living root and stem tissues.

Relative Toxicity of Mercuric Chloride and Silver Nitrate to *Ophiobolus graminis* and *Helminthosporium sativum*

Many investigations have shown that the toxicity of certain chemicals to fungi and bacteria varies greatly with the organism, the differences being so great as to form a basis for differentiation between certain species (6, 7).

**Since this section of the work was completed Mead (8) has published some studies of methods of treating diseased wheat roots and seeds for the isolation of fungi. He included silver nitrate in his study. However, owing to variations in technique employed, his results are not strictly comparable with those reported here.*

Coons and Strong (4) found that certain fungi were capable of growing in concentrations of copper sulphate which were decidedly toxic to other fungi. Link (7) was able to differentiate between *Fusarium oxysporum* Schlecht and *F. trichothecioides* Woll. by the use of certain organic acids as growth inhibiting substances in the media on which the two fungi were grown. Leonian (6) showed that species of *Phytophthora* differ in their ability to grow in media containing different concentrations of Malachite green, and Hotchkiss (5) found varying responses in the growth of bacterial cultures on media to which the chlorides of various cations had been added. Similarly, Bateman (1) found that *Fomes annosus* (Fr.) Cooke was more sensitive to mercuric chloride than to silver nitrate.

Having demonstrated that the chance of success in isolating *Ophiobolus graminis* was far greater when silver nitrate was used as a surface sterilizing agent than when mercuric chloride was used, and having observed that the latter chemical could be used successfully in the isolation of *Helminthosporium sativum*, the writer believed that the explanation might be found in the relative toxicity of these chemicals to the fungi in question. Consequently experiments were made to determine the effects of these chemicals in dilute concentrations on the growth rates of these fungi on artificial media.

To determine the relative toxicity of mercuric chloride and silver nitrate to *Ophiobolus graminis* and *Helminthosporium sativum* the fungi were grown in petri dishes on potato-dextrose agar containing the chemicals in concentrations ranging from 1 : 1,000 to 1 : 50,000. The diameters of the colonies were measured after five days' growth at room temperature. The results are given in Table IV and illustrated in Figs. 1 and 2.

It is evident that mercuric chloride is much more toxic to *Ophiobolus graminis* than to *Helminthosporium sativum*. This in itself might offer an explanation of the difficulty of isolating *O. graminis* from tissue previously surface

TABLE IV

EFFECT OF MERCURIC CHLORIDE AND SILVER NITRATE ON THE GROWTH OF *Ophiobolus graminis* AND *Helminthosporium sativum* ON NUTRIENT AGAR

Concentration of chemical added to the media	Diameters of colonies*							
	In mm.				In per cent of check			
	Silver nitrate		Mercuric chloride		Silver nitrate		Mercuric chloride	
	O. gram.	*H. sat.*	*O. gram.*	*H. sat.*	*O. gram.*	*H. sat.*	*O. gram.*	*H. sat.*
Check	30	64	24	67	100	100	100	100
1 : 1,000	trace	0	–	–	<1	0	–	–
1 : 5,000	21	7	0	23	70	11	0	34
1 : 10,000	31	24	0	37	103	38	0	35
1 : 20,000	–	–	trace	56	–	–	<1	84
1 : 50,000	30	61	4	62	100	95	17	93

**Average of 3 colonies except in the check which is the average of 6.*

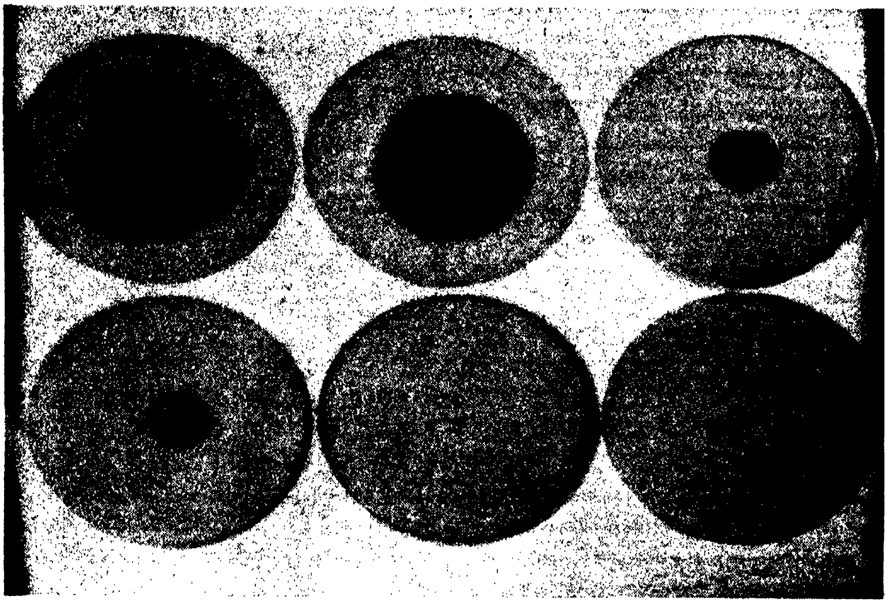

FIG. 1. *Reactions of Helminthosporium sativum (top row) and Ophiobolus graminis (bottom row) to different concentrations of mercuric chloride.*

Check *1 : 20,000* *1 : 5,000*

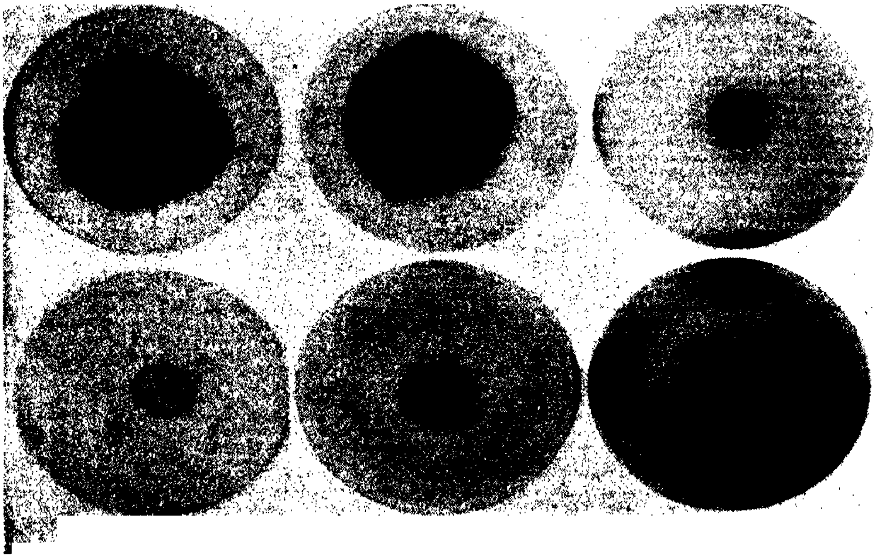

FIG. 2. *Reactions of Helminthosporium sativum (top row) and Ophiobolus graminis (bottom row) to different concentrations of silver nitrate.*

Check *1 : 10,000* *1 : 5,000*

sterilized with this chemical. Table IV also shows that *O. graminis* tolerates silver nitrate, at all concentrations tested, better than does *H. sativum*. This difference is especially marked at the concentration of 1 : 5,000, where the growth of *H. sativum* is only 11% of its check, whereas the growth of *O. graminis* is 70% of its check. These tests were repeated and gave almost identical results.

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RECENT INVESTIGATIONS ON TOBACCO ROOT ROT IN CANADA[1]

By L. W. Koch[2]

Abstract

These investigations involved the direct microscopic examination of root systems of tobacco plants showing typical macroscopic evidence of infection by *Thielaviopsis basicola* (Berk.) Ferraris, supplemented by isolations from both diseased and healthy roots and infection experiments with the isolates obtained. Intensive microscopic examination of lesions on approximately 1600 roots affected with typical black root revealed the presence of many different organisms which were found to occur singly, in various combinations with one another and more especially in frequent association with *T. basicola*. In addition to *T. basicola* the organisms observed included the following: the so-called phycomycetous type of "mycorrhizal" fungus, representatives of the genus *Rhizoctonia*, including *R. Solani* as well as several endophytic forms of the type familiar in orchids, different members of the genus *Pythium*, and nematodes. In general, members of the Fungi Imperfecti appeared to be conspicuously absent. Microscopic evidence of parasitism on the part of each of the above-mentioned organisms was revealed by necrosis involving single cells, groups of cells or entire rootlets. The phycomycetous "mycorrhizal" fungus resembling in many respects the fungus reported as being almost universally present in the roots of strawberry, immediately upon penetration produces characteristic coils of mycelium in the outer cortical cells, ramifies deeper, and spreads producing first arbuscules and then vesicles in the deeper cortical tissues. Previous investigators (11, 15, 20, 24) have reported this or a closely related form of the fungus only in the roots of host plants. In the present paper it is reported for the first time completely invading the stems and leaves of moss plants, also the thallus of liverworts found growing in muck soil obtained from tobacco seedbeds. Daily microscopic examination of tobacco seedlings developing in muck known to contain the phycomycetous "mycorrhizal" fungus revealed the presence of the latter organism in 30% of the roots as early as five days after germination and in all of them 10 days after germination.

Isolation from 206 typically diseased roots has consistently yielded *T. basicola* as well as representatives of 21 genera of fungi, also bacteria and nematodes. The fungi isolated most frequently in association with *T. basicola* included representatives of the genera *Pythium*, *Rhizoctonia* and *Fusarium*. Preliminary infection experiments have demonstrated that one *Rhizoctonia* of the "*Solani*" type and three endophytes from the same genus; four forms of *Pythium* as well as *T. basicola* possess parasitic capability in the roots of tobacco, whereas two other forms of *Pythium*, three of *Rhizoctonia* and seven distinct members of the Fungi Imperfecti showed no capability of parasitism of the same host under identical conditions.

Introduction

In Quebec, where cigar leaf and pipe tobaccos are chiefly grown, black root rot of tobacco has for years been the most serious disease of this host. In Ontario the situation varies. In the older tobacco-growing districts of Essex and Kent counties where Burley and dark types of tobacco are grown chiefly, root rot is of major importance, while on the other hand, in the newer district such as Norfolk county where the more resistant flue-cured varieties are grown the disease is of relatively little importance. As a result of a tobacco disease survey initiated in 1933 by the St. Catharines laboratory, investiga-

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tions on both fundamental and control phases of the black root problem were begun in the following year. In the present article are embodied results of the fundamental investigations of the problem to date.

Review of Literature

Since Peglion (17) in 1897 in Europe, and Selby (22) in 1903 in America, reported *T. basicola* as the primary causal organism of black root of tobacco an extensive literature dealing with all phases of the problem has appeared. Since the general literature has been well reviewed by previous investigators no repetition will be made here. However, during the past decade information concerning more particularly host reaction and the habits of *T. basicola* both in the soil and on the host has increased rapidly and mention will be made of a few outstanding contributions along these lines. Conant (6) in 1927 showed a distinct correlation between resistance to invasion and cork formation in the tissues underlying the lesions. He found resistant varieties capable of effectually "corking out" the fungus at 20°, 25° and 30° C. In the susceptible strains, however, there was no cork formation at 20° C. but effective corking out at 30° C. Earlier investigators had shown that the amount of root rot in the field depended primarily upon the prevailing soil temperature. More recently the intensive investigations of Doran (8, 9), and Anderson and co-workers (1–3) have shown that *T. basicola* is also sensitive to soil acidity, pH values of 5.9 or higher being favorable for the growth of the fungus. Doran has also contributed much valuable information concerning the control of this disease by the use of acetic, pyroligneous and other acids (7, 10). Within recent years considerable progress has been made in various Tobacco Experiment Stations in the breeding of varieties resistant to root rot. Numerous varieties have been, and are now being produced combining necessary commercial qualities with resistance to *T. basicola.*

Method of Investigation and Materials Used

Investigations of root rot of tobacco in the past have been concerned entirely with *T. basicola*, the effect of the pathogen on the host having been studied in most instances by infection experiments in sterilized soils. In the present investigation direct microscopic examination of diseased roots growing under varying *natural* conditions both in the seedbed and in the field has been relied upon to give an accurate picture of black root of tobacco as it actually exists in nature. To supplement these studies, isolations were made from carefully selected material and, using pure cultures of the organisms obtained, infection experiments were conducted to test their pathogenicity.

Specimens of diseased plants were supplied as required, by the Dominion Experimental Stations at Farnham and L'Assomption in Quebec, and Harrow in Ontario. In addition, a supply of diseased material was always available from the seedbeds at the St. Catharines laboratory where seedlings were grown in virgin muck soil with *T. basicola* in pure culture added in some cases.

Microscopic Examination of Roots of Tobacco Affected with *T. Basicola* (Berk) Ferraris

In the microscopic studies, in addition to the materials mentioned above, tobacco seedlings grown in flats in the greenhouse were also examined daily *in toto* from the time of germination. Altogether, approximately 1600 roots of all ages were examined and with the exception of larger roots, where razor sections were made, the roots were prepared for examination by the method employed by Hildebrand (11) and Truscott (24) in their studies of the roots of strawberry. When the preparations were stained too deeply, entirely satisfactory results were obtained by de-staining slightly in clear lactophenol heated almost to boiling.

By this method it is possible to identify with accuracy in tobacco roots even different members of the same genus, particularly, for example, *Rhizoctonia* and *Pythium*. In relatively infrequent instances the occurrence of septate mycelium possessing no further diagnostic characteristic made identification impossible.

The organisms which were observed to occur singly, in association with one another, or with *T. basicola* will be dealt with separately.

THE PHYCOMYCETOUS "MYCORRHIZAL" FUNGUS

An endophyte of the phycomycetous type of mycorrhizal fungus in many respects similar to the organism present in strawberry roots first described in detail in that host by O'Brien & McNaughton (19) and reported more recently by Hildebrand (11), Truscott (24) and Berkeley & Lauder-Thomson (4) was observed almost invariably in the roots of tobacco. It was associated in lesions with *T. basicola* more frequently than any other organism. During the course of his investigations on mycorrhiza, Peyronel (20, 21) in Italy included tobacco in his long list of plants in the roots of which he observed the phycomycetous "mycorrhizal" fungus. Since there appears to be no record of this fungus as it occurs in tobacco roots in America it is described below with a discussion of its relation to root rot of tobacco.

The characteristic, coarse, irregular and generally non-septate mycelium has been found to be particularly abundant in the unsterilized muck soil used for growing tobacco seedlings. This mycelium frequently gives rise to extremely fine lateral branches in which numerous septations have often been observed. Strands of the coarse mycelium may adhere closely to the epidermal cells of any portion of the root surface with the exception of the extreme tip, frequently spreading out over the surface at point of contact. After effecting penetration at a given point the fungus continues to grow externally in the direction of the long axis of the root, and again making contact with the root surface, forms another mycelial weft. This may be repeated many times, thus producing multiple infection. As a rule hyphae from these mycelial wefts penetrate the epidermal tissue intercellularly but sometimes directly through the cell wall. Immediately after entry the mycelium gives rise to a distinct and characteristic coil within the epidermal cell. Similar coils are also often produced in adjacent and immediately underlying cells (Plate III, 4). In this respect the fungus differs from the organism of the same type found in strawberry. Hyphal strands ramify the underlying cortical tissue both inter- and intracellularly advancing more rapidly tangentially. Immediately after invasion of the cortical cells where coiling of the mycelium also occurs, though to a lesser extent than in the epidermal tissue, arbuscules appear (Plate I, 5, 6). The arbuscules which are similar in character to those produced by the fungus in strawberry roots appear to function as haustoria in their earlier stages and later are partially digested by the host cells. Subsequent to the formation of arbuscules, vesicles appear (Plate I, 1, 2, 4). For the most part the vesicles are intercellular though intracellular vesicles have also been observed. The vesicles of the organism in tobacco roots are typically sphaeroidal (Plate II, 1) and in this respect also differ from the fungus found in strawberry roots (11, 24). In numerous cases distortion of the root was observed as a result of the abundance of the vesicles. In other cases where mycelium alone was abundant, a constricted lesion resulted. Moreover the roots were always more brittle in the region of heaviest infestation and frequently broke at these points during manipulation.

Starch is absent in the cells of tobacco roots permeated with the fungus. In some cases no discoloration or death of cells has been observed in infected tissue. On the other hand necrosis involving single epidermal cells containing coils of mycelium, or groups of cells has been observed both in seedlings and in more mature plants heavily invaded by this fungus alone. The vesicles which are at first thin walled and the contents of which appear finely granular gradually develop a thicker wall and the contents become vacuolate. Occasionally vesicles have been observed which appear to be parasitized by a fungus of the *Rhizoctonia* type (Plate I, 1).

It is interesting to note that roots of tobacco plants from a certain field near Simcoe, Ontario where *T. basicola* appeared to be entirely absent were affected with brown root. Examination of these roots revealed a surprising abundance of the phycomycetous "mycorrhizal" fungus and an almost total absence of other fungi.

The same fungus was commonly observed growing in the muck of the St. Catharines seedbeds. Some of this muck soil was transferred to flats in the early fall and the latter were removed to the greenhouse, and tobacco seed was sown in them. A species of moss as well as a species of *Marchantia* common to muck soils partially overgrew the surface of these flats. Later, dying moss plants as well as a few diseased thalli of *Marchantia* were observed. Microscopic examination of these plants revealed the mycelium of the phycomycetous "mycorrhizal" fungus with an abundance of vesicles ramifying in both the stems (Plate III, 1) and leaves of the moss plants and the thalli of *Marchantia*, mycelium and vesicles extending to the growing tips of the plants (Plate I, 3). An examination of Plate I, 3 and Plate III, 1 will show that in the moss plants the spherical vesicles characteristic of the organism in tobacco predominated.

In February, moss plants were obtained directly from the outdoor seedbeds and in certain of them the same mycorrhizal organism was also present.

These findings would indicate one means by which this organism can overwinter in tobacco seedbeds. It is believed that this is the first report of the presence of this phycomycetous "mycorrhizal" fungus in the aerial parts of plants.

To find out when tobacco seedlings may become infected by the phycomycetous "mycorrhizal" fungus, seeds of tobacco, variety Judy's Pride, were sown during the summer of 1934 in muck known to be heavily infested with the organism. Daily, from the time of germination ten seedlings were removed and examined microscopically. The results obtained appear in Table I.

TABLE I

RESULTS OF MICROSCOPIC OBSERVATION OF TOBACCO SEEDLINGS GROWING IN MUCK SOIL

Days after germination	Organisms present and percentage occurrence in roots				
	phycomycetous "mycorrhizal" fungus	*T. basicola*	*Rhizoctonia*	*Pythium*	Other fungi
5	30	—	—	—	—
6	80	10	10	10	—
7	90	30	—	10	—
8	100	60	20	20	10
10	90	20	10	10	10
13	100	40	30	30	30

It will be observed from an examination of Table I that five days after germination 30% of the seedlings were already infected by the phycomycetous "mycorrhizal" fungus and eight days after germination 100% had become infected. It is also interesting to note in this connection that six days after germination the seedlings showed primary infection by representatives of at least four different genera of fungi.

THE GENUS *Rhizoctonia*

Rhizoctonia Solani has been reported frequently in the past as the cause of damping-off of tobacco seedlings (5, 12–14, 18) and also in association with "sore shin" (13, 14), a characteristic root trouble of older tobacco plants in the field. *R. bataticola* has also recently been reported (25) causing the latter disease in Kentucky. During the present investigations representatives of the genus *Rhizoctonia* have been observed associated with *T. basicola* in the same lesions (Plate II, 3) especially in diseased plants from Quebec. However, *Rhizoctonia* has not been observed as frequently in diseased roots as the phycomycetous "mycorrhizal" fungus. Different forms of *Rhizoctonia* can be readily distinguished by microscopic examination. *R. Solani* is easily recognized by the extremely coarse, septate mycelium with its characteristic branching and frequent anastomosis. On young seedlings the coarse hyphae effect penetration at or near the ground level and ramify in the cortical tissues of the root and stem causing the typical and well known symptoms of damping-off. In older plants which have not succumbed to typical damping-off, an abundance of superficial mycelium is often observed adhering to the root surface. Hyphae from this superficial mycelium penetrate cortical cells, producing in them pseudo-sclerotial complexes.

Infection of both seedlings and older roots by forms other than *R. Solani* is of much more frequent occurrence. In general, these forms give rise to sclerotia-like bodies which may occupy single cells or groups of cells in the cortical tissues and from which extends typical *Rhizoctonia* mycelium much smaller in diameter than that of *R. Solani.* These sclerotia-like bodies vary considerably, some consistently occupying only part of a cell whereas others completely fill the cell. Certain of them correspond closely to the monilioid bodies characteristic of the "orchid" type of *Rhizoctonia* (Plate II, 4) as described by Peyronel (21) and others. All degrees of infection by *Rhizoctonia* were observed both in close association with *T. basicola* and alone, and producing varying degrees of visible necrosis.

THE GENUS *Pythium*

Pythium has frequently been reported as a cause of damping-off of tobacco seedlings (5, 12, 14). As in the case of *Rhizoctonia*, *Pythium* was frequently observed during the present investigations in lesions on the younger roots of tobacco plants beyond the stage at which damping-off usually occurs. The representatives of this genus were observed alone (Plate II, 5, 6, 7), in combination with other organisms or in association with *T. basicola* (Plate II, 2). Microscopically the members of this genus in root tissues can be identified as such either by the presence of thin-walled conidia or zoosporangia, or as is more frequently the case, by the thick-walled oospores (Plate II, 5). Only in very early stages of infection is mycelium found in lesions. There is a wide variation in the type and size of spores observed, also a great difference in degree of necrosis, which suggests the presence of different members of the genus.

THE GENUS *Asterocystis*

Resting spores of *Asterocystis*, probably *Asterocystis radicis*, de Wild, were frequently observed in diseased roots of both seedlings and older plants in the field, particularly the latter (Plate III, 6 and 7). Peyronel in his investigation on mycorrhiza (20) observed *A. radicis* on both healthy and decayed roots of numerous plants and expressed the opinion that under conditions unfavorable to certain hosts the organism could become parasitic. Vanterpool (26) in 1930 concluded that only under exceptionally unfavorable conditions would *A. radicis* cause significant damage on cereals, and then only on oats. Truscott (24) in 1934 concluded this organism to be an important pathogen on strawberry roots under moist conditions.

In roots of tobacco this organism, which is readily identified under the microscope, was observed both alone and in combination with *T. basicola* or other organisms. In most tissues of tobacco roots where *Asterocystis* was observed, this fungus did not appear to cause much injury though in some cases where spores were particularly abundant, for example in plants grown in heavier soils, necrosis was frequently observed. It was concluded, therefore, that in ordinary soils under normal conditions little or no damage is caused by this organism but that in heavier soils, under conditions unfavorable to the host, this fungus may become a primary parasite or may be one of a complex of organisms causing a root rot.

Thielaviopsis basicola, (Berk.) Ferraris

As was stated previously this organism has been recognized for many years as a virulent parasite on roots of tobacco and consequently no discussion of its parasitism will be made here. However, a point in connection with its morphology which apparently has not been pointed out by previous investigators may be mentioned here, namely, that though this fungus in common with many others possesses septate mycelium, its hyphae in the root cells are sufficiently characteristic to render diagnosis certain. The younger advancing hyphae have the appearance of a chain of crescent-shaped spores, the septa occurring at the constrictions in the chain. Penetration of cell walls is effected by thread-like hyphae which after penetration develop first a spear-like head followed by a chain of the normal, crescentic segments (Plate III, 5).

NEMATODES

The nematode *Heterodera marioni* (Cornu) Goodey has frequently been reported as causing galls on the roots of tobacco (23, 27), and *Anguillulina pratensis* (de Man) Goffart, has also been reported in association with a brown root of tobacco in alkaline soils (16). During the course of the present investigations, nematodes of a type distinctly different from *Heterodera*, were frequently observed alone or in black root lesions. When alone in the root tissues they are probably of no economic importance. Nevertheless, dead or dying cells or groups of cells were frequently observed surrounding single nematodes (Plate III, 2, 3). In such cases these dead cells might easily pave the way for certain facultative parasites which in the absence of infection courts would play no part in root rot. In all cases nematodes or their eggs were observed only in the cortical tissues of the roots.

Isolations from the Roots of Plants Affected with *T. Basicola*

In making isolations, individual diseased rootlets were first thoroughly washed under running water with a camel's hair brush and then transferred to Petri dishes containing potato dextrose agar (2½% dextrose), at the rate of five or six per plate. Apparently healthy rootlets from the same plants were treated in a similar manner. In some cases the medium was acidified, but in most instances non-acidified medium was used to permit the development of Phycomycetes. As the fungi grew beyond the edge of the bacterial colonies single hyphal tips were cut off and removed to tubes. In nearly all instances more than one fungus was isolated from a diseased rootlet. Owing to the difference in growth rates of the various fungi it was sometimes possible to obtain all of them in pure culture while at other times this proved to be impossible. Frequently after the plantings were a week or more old, by which time the plate was usually overrun with numerous fungi, those which had developed more slowly produced spores near the diseased rootlet, by which they were identified. The results of isolations from 206 diseased rootlets and from 36 apparently healthy rootlets from the same plants are summarized in Table II.

TABLE II

ORGANISMS ISOLATED FROM LESIONS ON ROOTS OF TOBACCO SHOWING MACROSCOPIC EVIDENCE OF INFECTION BY *T. basicola* AND FROM APPARENTLY HEALTHY ROOTS OF THE SAME PLANTS

Organism	Number of isolates obtained		
	From Quebec materials	From Ontario materials	From apparently healthy roots
Thielaviopsis basicola	48 (2)*	17	3
Rhizoctonia spp.	36 (7)	13	3
Pythium spp.	18 (10)	28	1
Fusarium	31 (7)	11	17
Penicillium	6 (4)	3	1
Alternaria	4	1	3
Coniothyrium	6	2	Nil
Phoma	3	Nil	Nil
Pestalozzia	1	Nil	Nil
Hormodendrum	3	1	4
Cladosporium	2	Nil	Nil
Trichoderma	17	12	11
Mucor	11	7	4
Cephalosporium	2	Nil	Nil
Asterocystis	6	1	Nil
Nematodes	Fairly abundant	Sparse	Nil
Bacteria alone	3	Nil	1
Hainesia	3	Nil	Nil
Dematium	4	2	2
Verticillium	2	1	Nil
Yeast-like fungi	4 (4)	5	2
Rhizopus	6	5	7
Cephalothecium	2	Nil	Nil
Unidentified fungi	4	Nil	Nil

Numbers in brackets are the numbers of morphologically distinct strains isolated.

An examination of Table II shows that representatives from at least 21 genera of fungi as well as bacteria and nematodes were isolated from diseased rootlets selected because of macroscopic evidence of infection by *T. basicola.* It is true that *T. basicola* appeared most frequently in culture as would be expected, but isolates of ***Rhizoctonia***, ***Pythium*** and ***Fusarium*** also occurred very consistently. ***Rhizoctonia*** was isolated most frequently from diseased specimens from Quebec, while ***Pythium*** appeared most frequently from the roots of plants from Ontario. It will also be observed that *Asterocystis* appeared on seven occasions. The occurrence of this fungus in the plates was in the aggregate probably much more frequent than the tabulated results would indicate because during the earlier part of the work this organism, though present, was not identified. It is well known that *Asterocystis* is an obligate parasite and as such cannot be obtained in pure culture. However, in the instances mentioned above, the organisms appeared in abundance in the medium surrounding the diseased tissues as early as 24 hours after the tissue plantings were made and before other fungi had developed. It is possible that under favorable conditions of temperature and moisture, zoospores were produced by, and set free from, the resting spores within the root tissues, and these zoospores spreading over the surrounding medium gave rise to an abundance of mature spores of the fungus.

While fewer isolations were attempted from healthy roots it will be noted that there was almost complete overlapping from the standpoint of organisms isolated. However, this is to be expected when isolations are made from delicate tissues which cannot stand surface sterilization and which cannot be washed perfectly. In any case a low percentage of individual organisms was isolated from the apparently healthy roots, with the exception of *Fusarium*, *Trichoderma*, and possibly *Rhizopus*. To summarize the results of all isolations, the most noteworthy feature was the frequent appearance of both *Pythium* and *Rhizoctonia*. Their relative absence from the healthy rootlets would indicate that they, as well as *T. basicola*, are pathogens. It is already well known that both *Pythium de Baryanum* and *Rhizoctonia Solani* cause damping-off of tobacco seedlings, but none of the roots from which the isolations were made showed symptoms of damping-off and in most instances the plants used were beyond the stage when damping-off occurs. It will also be noted that seven morphologically distinct strains of *Rhizoctonia* were isolated. Only one of these was identical with *R. Solani* while four of the remaining six possessed characters as described by Peyronel (21) for the "orchid" type of *Rhizoctonia*. Similarly, ten morphologically distinct strains of *Pythium* appeared in the isolations. It may seem surprising that *T. basicola* did not appear more frequently but this is readily explainable when it is recalled that *T. basicola* is a relatively slow growing organism and as such cannot successfully compete with the numerous fast growing organisms that so frequently are associated with it in lesions on the host.

In view of the frequent appearance of *Fusarium*, *Trichoderma* and *Rhizopus* from apparently healthy roots, these organisms may be regarded as non-pathogenic.

Preliminary Infection Experiments

Preliminary infection experiments on tobacco involving 28 different fungi representing 11 genera as follows: *T. basicola* (2 strains), *Rhizoctonia* (6 strains), *Pythium* (7 strains), *Trichoderma*, *Alternaria*, *Fusarium* (6 strains), *Mucor*, *Cephalosporium*, *Verticillium*, *Coniothyrium* and *Pestalozzia* were conducted. Inoculum was prepared by growing each organism on sterilized crushed oats in 250 cc. Erlenmeyer flasks, the contents of each flask being sufficient to inoculate three 5-inch pots containing steam sterilized soils. Check pots to which sterile oats were added in the same proportion were also prepared. Tobacco seed was sown in all pots and the roots of seedlings were examined microscopically for evidence of infection.

Certain of the organisms manifested primary parasitism (Fig. 1) which ranged from (a) invasion of host tissue without killing, *e.g.*, one strain of the "orchid" type of *Rhizoctonia* and one form of *Pythium*, through (b) local necrosis, *e.g.*, one strain of *Pythium*, to (c) general destruction of the complete root system, *e.g.*, *T. basicola* (2 strains); *Rhizoctonia* ("orchid" type, 2 strains) *R. Solani; Pythium* (3 strains). Negative results were obtained with all the other organisms mentioned above. Though all pathogenic strains of *Pythium* and two of the four strains of *Rhizoctonia* caused some measure of damping-off, all of them proved capable of causing definite root rot both in seedlings and in older plants.

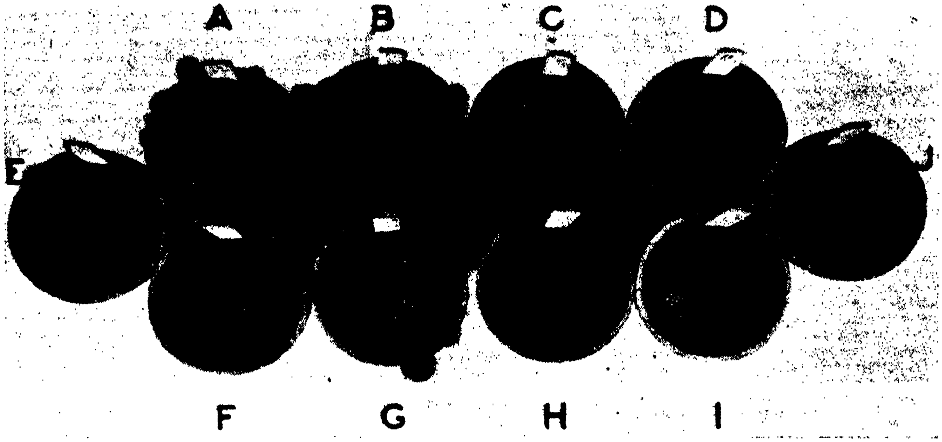

FIG. 1. *A, B, C and D—checks; E, F, G, H, I and J—inoculated. Soil in E and F each inoculated with a different strain of Pythium; A and B corresponding checks. Soil in G, H, I and J each inoculated with a different strain of Rhizoctonia; C and D corresponding checks.*

It should be emphasized that the results of these infection experiments closely confirmed the evidence obtained (a) from microscopic examination of and (b) isolations from carefully selected roots showing evidence of black root.

Discussion

For many years *T. basicola* has been recognized as a virulent parasite on the roots of tobacco, and its pathogenicity has been repeatedly demonstrated by inoculation of plants in sterile soil. On hosts other than tobacco specific primary root parasites have also been demonstrated, *e.g.*, *Gibberella Saubinetii* (Mont.) Sacc., on corn and wheat, *Ophiobolus graminis* Sacc., on cereals *Phymatotrichum omnivorum* (Shear) Duggar, on cotton, as well as many others. On the other hand a somewhat different situation exists in the case of certain other root rots, notable among which is strawberry root rot. In recent years the latter disease has received considerable attention. O'Brien and McNaughton (19) in Britain attributed the "Lanarkshire" root rot of strawberries to a phycomycetous mycorrhizal organism. Berkeley and Lauder-Thomson (4) in England demonstrated the pathogenicity of five members of the Fungi Imperfecti on roots of strawberries, and Truscott (24) in Canada proved by infection experiments on the same host the pathogenicity of members of seven widely separated genera of fungi, and obtained microscopic evidence of parasitism on the part of three additional obligate parasites of the phycomycetous type.

Black root rot of tobacco is more analogous to the first mentioned type of root rot, especially since a single virulent parasite is primarily responsible, and also because tobacco is an annual crop while strawberries are perennial. However, notwithstanding the fact that *T. basicola* is the primary causal agent of the disease which has come to be known as black root rot of tobacco, heretofore infection experiments involving *T. basicola* have been carried out using sterilized soil and therefore a true picture of black root rot as it actually exists in the field or in unsterilized seedbeds has not been presented. Of outstanding importance in the present investigations is the fact that numerous other organisms are indisputably associated with *T. basicola* in black root rot of tobacco. This conclusion was reached as a result of complete examinations of root systems (possible only by the use of a satisfactory staining and clearing technique) of seedlings and more mature plants growing in seed bed and field soils which produced typical black root rot. The results obtained from isolations and subsequent infection experiments supplemented and confirmed the evidence adduced from microscopic examinations.

Almost invariably other organisms, also capable of destruction of roots, were present either on the same lesions with *T. basicola* or on other rootlets of the same plant infected by this organism. Roots from the seedbed or field exhibiting macroscopic evidence of black root rot due to the presence of chlamydospores of *T. basicola* on the lesions reveal on close examination the presence of brown lesions also. Some of these are incipient infections by *T. basicola* on which chlamydospores have not yet developed, but many others are due to infections by one or more other organisms in various combinations.

Direct microscopic examination of a large number of roots of all ages has revealed the fact that a species of *Asterocystis* and an organism resembling the so-called mycorrhizal fungus reported as being almost invariably present in the roots of strawberry, possess parasitic capabilities on the roots of tobacco. Five morphologically distinct representatives of the genus *Pythium* and four isolates of *Rhizoctonia*, all of which were obtained from diseased roots showing infection by *T. basicola* have been proved by infection experiments to be each capable of primary parasitism on roots of tobacco. Several members of each of the above genera also caused damping-off, which phenomenon is in accordance with previous conceptions regarding members of these genera. In the present investigation, however, their importance was considered only in the light of their capability of causing root rot. Numerous other organisms also isolated from roots infected with *T. basicola* proved in preliminary infection experiments to be non-pathogenic on tobacco. *T. basicola* as well as the other pathogenic organisms appeared in all possible combinations in both brown and black lesions. An evaluation of the importance of each of these organisms in root rot of tobacco is extremely difficult.

It is well known that during cool, wet seasons injury from *T. basicola* is most severe and in such a season it is also likely that *Pythium* would be abundant. In any case it would seem to be interesting that in a root rot disease considered heretofore to be caused strictly by one parasite, other organisms capable themselves of primary parasitism of the host should be so constantly associated with the chief causal organism, *T. basicola*, in this case. It is quite possible that investigations along similar lines of other root rot diseases analogous in this respect to black root rot of tobacco will yield similar results. From the standpoint of control the present results do not change the situation materially in respect to seedbeds because when seedbed soil is thoroughly sterilized all organisms are killed.

In the field, loss of plants is overcome by the use of resistant varieties. It is a well known fact that where a given crop is grown intensively within a given area, sooner or later epiphytotics develop. *T. basicola* has already in Quebec threatened to become a limiting factor in the growth of tobacco and the loss of plants, as previously stated, is only overcome by the use of plants resistant to *T. basicola*. There is no assurance, however, that these varieties which at present are resistant to *T. basicola* will also prove indefinitely resistant to certain of the other organisms which have been shown capable of primary parasitism, especially in view of the possibility of a constantly changing microbiological balance in the soil.

In concluding, it should be mentioned that the possibilities of investigating root rot of tobacco by the present method of attack are by no means exhausted. It is now planned to make daily microscopic examinations and comparisons of the complete root systems of seedlings and more mature tobacco plants growing under different environmental conditions to obtain as complete a picture as possible of the progress of infection by different organisms as it occurs in nature.

Acknowledgment

Thanks are gratefully extended to Dr. G. H. Berkeley for helpful suggestions offered during the course of this investigation, and to Messrs. H. F. Murwin and R. J. Haslam of Harrow, and Messrs. R. Bordeleau and G. E. Turcotte of Farnham and L'Assomption Experiment Stations respectively, for their generous assistance in supplying materials on numerous occasions.

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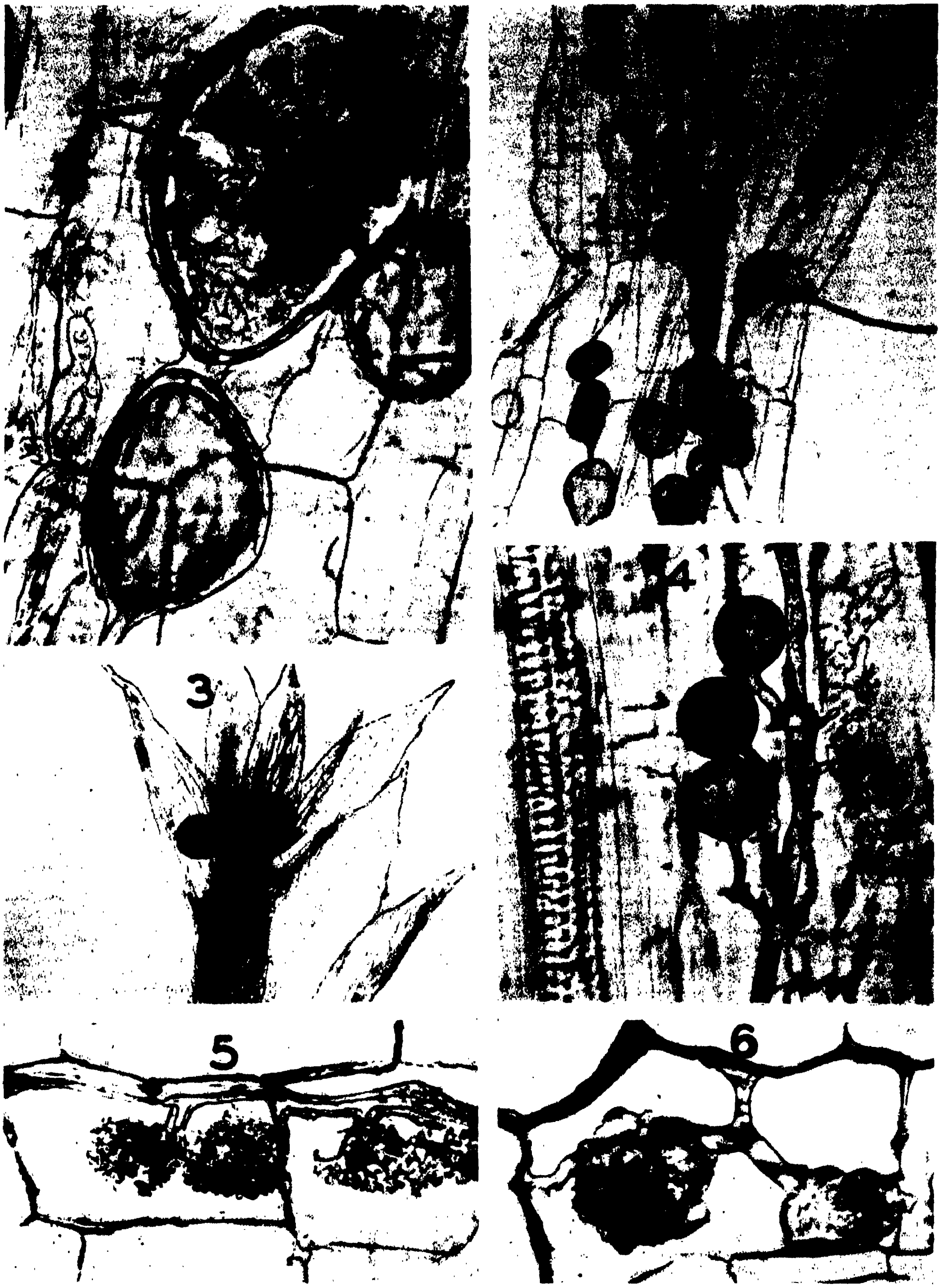
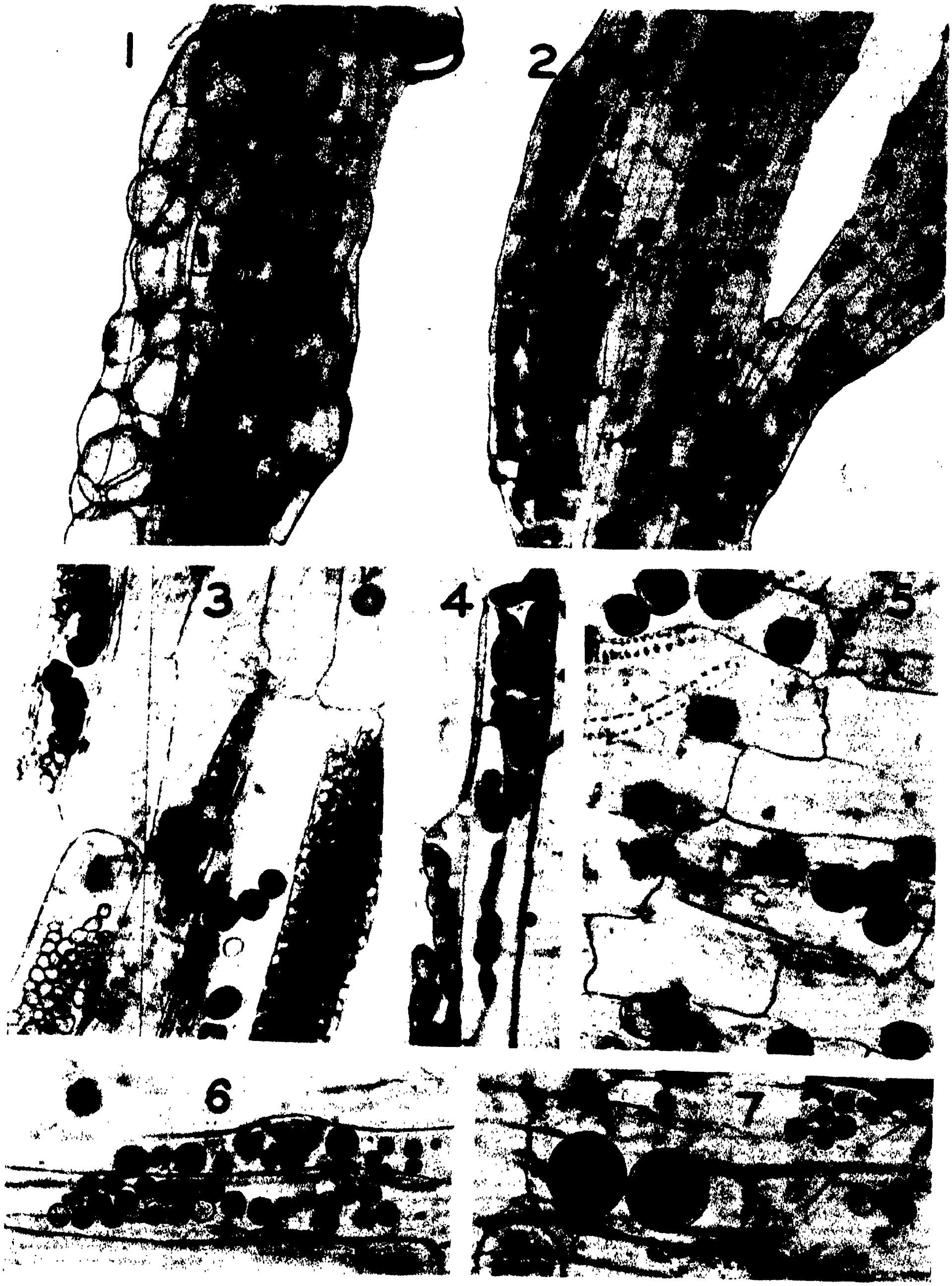
PLATE I

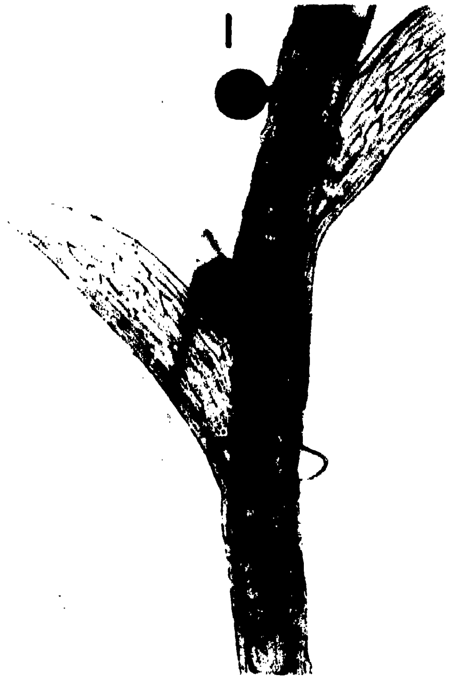


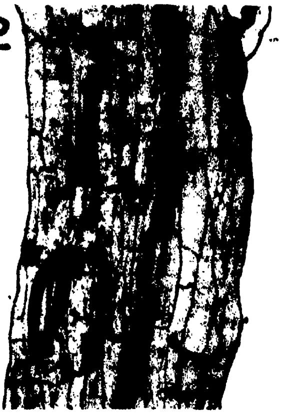
"*Phycomycetous mycorrhizal fungus*" (*photomicrographs*). *All figures except 3, in roots of tobacco.* FIG. 1. *Showing vesicles, the larger one of which contains Rhizoctonia-like mycelium,* ×*370.* FIG. 2. *Abundance of vesicles in root,* ×*80.* FIG. 3. *Vesicles at apex of moss plant,* ×*80.* FIG. 4. *Mycelium, from which both vesicles and arbuscules have arisen,* ×*340.* FIG. 5. *Arbuscules in early branching stage,* ×*340.* FIG. 6. *Later stage of 5 in "partially-digested" stage,* ×*340.*

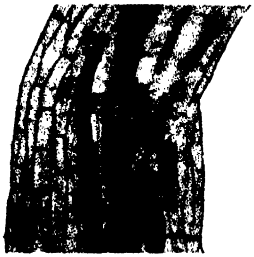
PLATE II

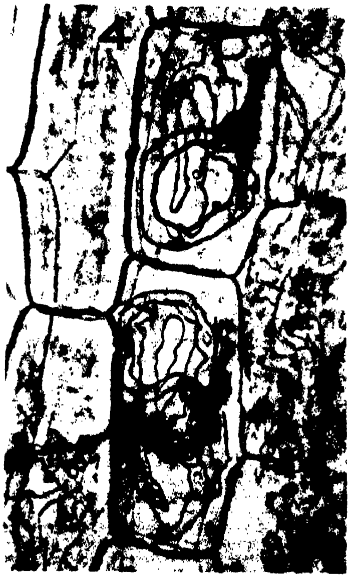


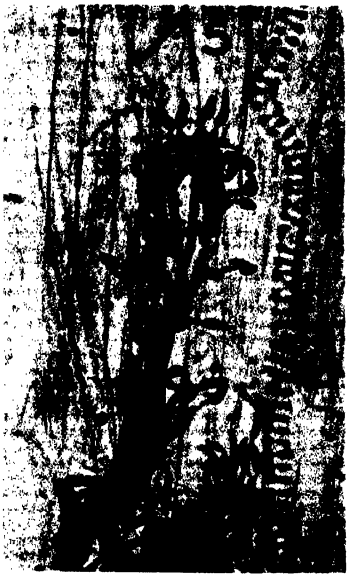
Photomicrographs. FIGS. 1, 2 AND 3. *T. basicola associated with other organisms in black root lesions. Fig. 1, with "phycomycetous mycorrhiza", ×340; Fig. 2, with Pythium, ×110; Fig. 3, with Rhizoctonia, ×340. Fig. 4. "Orchid" type of Rhizoctonia, ×340.* FIGS. 5, 6 AND 7. *Spores belonging to different forms of Pythium present in roots, ×340; Fig. 5, produced by inoculation.*

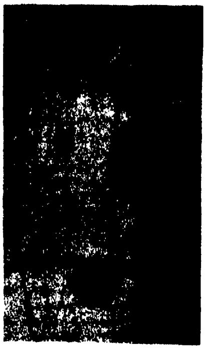


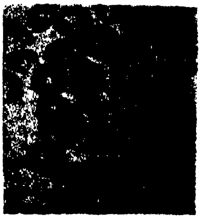












Photomicrographs. FIG. 1. *Vesicles of "phycomycetous mycorrhiza" throughout stem of moss,* ×*80.* FIGS. 2 AND 3. *Nematodes and eggs in lesions on roots,* ×*80.* FIG. 4. *Showing characteristic coils of mycelium of "phycomycetous mycorrhiza" in epidermal cell,* ×*340.* FIG. 5. *Characteristic method of penetration of cortical cells by T. basicola,* ×*340.* FIGS. 6 AND 7. *Asterocystis; Fig 6, Spores in root tissue,* ×*340; Fig. 7, Spores on surface of medium,* ×*340.*

THE OCCURRENCE IN NATURE OF MUTUAL AVERSION BETWEEN MYCELIA OF HYMENOMYCETOUS FUNGI[1]

By Harold J. Brodie[2]

Abstract

A specimen of *Corticium calceum* Fr. discovered in the herbarium of the University of Toronto is clearly an example of the barrage phenomenon in nature. Two mycelia differing both in color and texture grew toward one another on the wood substratum and between them there developed a clearly defined gap or barrage. The gap is plainly not the work of insects and judging from several characters is a true barrage developed under natural conditions. Careful search among fungi which form flat expanses of mycelium (such as the Thelephoraceae) may show that the barrage effect occurs commonly in nature.

Mycelia grown side by side on nutrient agar frequently develop between them a line of demarcation. Some kind of mutual aversion exists which causes the hyphae of one individual to avoid the zone occupied by the other. This reaction may be manifest between mycelia belonging to different species and genera and in some instances between mycelia derived from spores of the same species. The aversion has generally been ascribed to the metabolism of one mycelium causing the liberation of certain chemical substances which are unfavorable to the development of the other mycelium concerned.

In recent times, much research has been devoted to the analysis of the pairing reactions of single-spore cultures of many kinds of fungi. Occasionally it has been reported that the aversion between individual mycelia is not a haphazard occurrence but that it depends upon inherited characters.

The first critical analysis of the cause of mutual aversion was made by Vandendries (1) in 1931, who demonstrated that in *Pleurotus columbinus* aversion is perfectly correlated with genetic constitution.

The following year, Dr. Vandendries and the present writer (3), working in collaboration, undertook a more detailed study of the problem of aversion. Monosporous cultures of the wood-destroying fungus *Lenzites betulina* proved to be ideal material. The aversion of certain mycelia for one another was striking, in some instances producing a gap between them nearly one centimetre in diameter (Plate I, Fig. 1). To this mutual aversion the name *barrage* was given.

It was shown that the pairing reactions of monosporous thalli are governed by two pairs of allelomorphic factors, one of which (the *B* factor) is responsible for aversion. The aversion is entirely dependent upon the genetic constitution of the mycelia which are confronted with one another.

Vandendries and Brodie concluded that it is difficult to explain the results of their experiments by the assumption that the repulsive effect is of a chemical nature. The barrage seemed capable of manifesting itself between mycelia

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[1] *Manuscript received May 28, 1935.*
Contribution from the Department of Botany, University of Toronto, Toronto, Canada.

[2] *Fellow of the National Research Council (United States of America) at the University of Toronto.*

which were separated by a thin glass partition, and the degree of manifestation appeared to be dependent upon the nature and thickness of the partition. Further investigations by Vandendries (2) and independent researches by Brodie (unpublished) in part confirm the preliminary work and in part contradict it.

The details of this experimental work will be recorded elsewhere. The purpose of this communication is to report the finding of the barrage effect in nature.

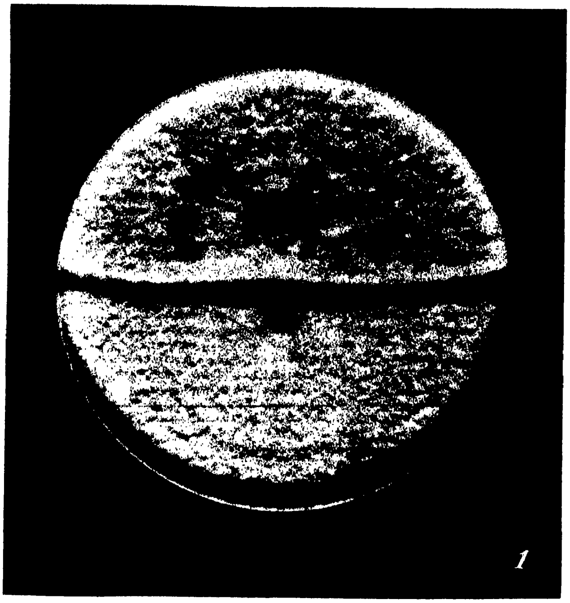
It had already been shown that in *Lenzites betulina* aversion can be developed between any two mycelia which differ in the *B* factor. If this condition is fulfilled, a monocaryophyte may repel either another monocaryophyte or a dicaryophyte, and two dicaryophytes may repel one another. In the Hymenomycetes, mycelia occurring in nature are usually dicaryophytic. Since in *L. betulina* dicaryophytes may repel one another, it appeared not unlikely that examples of the barrage effect might be found among specimens growing under natural conditions.

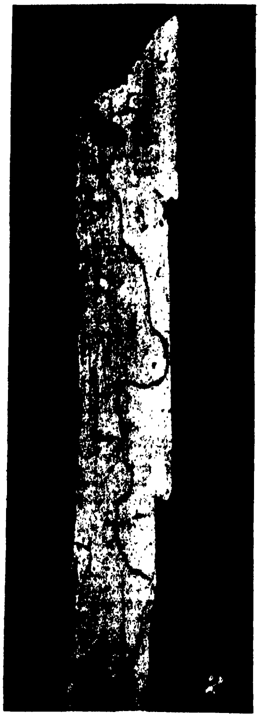
Professor H. S. Jackson, in discussing this point with the writer, mentioned that there was a specimen in the herbarium of the University of Toronto which resembled the barrages obtained on culture plates. The specimen had been noticed by Dr. R. F. Cain and had been discussed by him and Professor Jackson. Being unaware of the possibility of a barrage being formed between dicaryophytic mycelia, Professor Jackson had not considered the specimen of importance until discussing it with the writer.

The specimen (No. 6772 in the Herbarium of the University of Toronto) belongs to the thelephoraceous species *Corticium calceum* Fr. It is growing on a piece of rotted pine wood and was collected by Professor Jackson on Bear Island in Lake Timagami, Ontario, July 30, 1934. The yellowish fructification is thin and appressed, having a somewhat chalky appearance. Two mycelia, differing both in color and texture, have grown toward one another on the wood substratum. Between them is a clearly defined narrow gap (Plate I, Fig. 2) extending the entire length of the specimen (15 cm.). When examined under the microscope, the gap is found to be almost entirely devoid of hyphae (Plate I, Fig. 3). It has also many characters typical of the barrages seen in culture and there is every probability that it is a true barrage developed under natural conditions.

The facts which make such an assumption seem reasonable are as follows:

(*i*) The mycelium on one side of the barrage differs in color and texture from the mycelium on the other side. One mycelium is dark ivory in color, rough, and finely cracked; the other is pale ivory to whitish, smoother, and less cracked. Microscopic examination of sections taken from both sides of the barrage line shows that both mycelia belong to the same species. The difference between the thalli may be accounted for by assuming that one is older than the other. One mycelium had become established on the log. Another inoculation of the same fungus then occurred and the younger





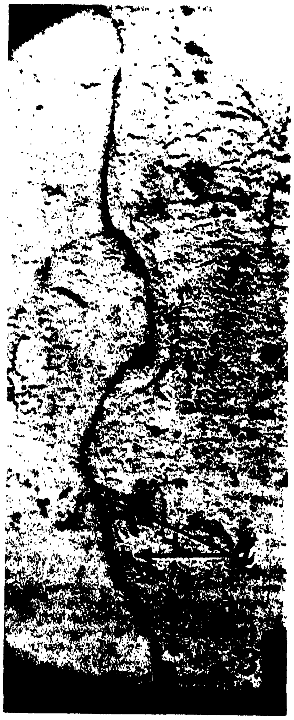


FIG. 1. *Barrage developed between two haploid mycelia of Lenzites betulina, mycelium No. 29 (AB) on the upper side of the barrage and No. 3 (Ab) on the lower side.* ×⅔.

FIG. 2. *Barrage developed in nature between two diploid mycelia of Corticium calceum Fr.* ×½.

FIG. 3. *Enlarged portion of the specimen of Corticium calceum shown in Fig. 2.; a, mycelium accumulated on one side of the barrage forming a "drift".* ×4.

mycelium grew toward the older until checked by the barrage action. On the other hand, the two mycelia may possibly have resulted from simultaneous inoculations and the differences in color and texture may be merely the differences between individuals. This seems less likely than the first hypothesis, as the differences are exactly those ordinarily noted between young and old mycelia of fungi belonging to the Thelephoraceae.

(*ii*) At certain points along the barrage line (Plate I, Fig. 3, *a*), mycelium from one side has accumulated to form a kind of drift such as is frequently seen on old culture plates where the vegetation accumulates and does not grow beyond an area delimited by the barrage action.

The two characters described discount the possibility of the gap between the mycelia being due to the ravages of a bark beetle or some other insect. Also there is a complete lack either of mycelial fragments or of excrement.

The specimen had been fumigated before inclusion in the herbarium and there was consequently no possibility of obtaining a culture of the fungus.

As far as the writer is aware, this is the first time that a barrage has been observed in nature. There is no reason why mycelia should not repel one another under natural conditions as they do under the conditions of artificial culture. Barrages to be noticeable would have to be developed under conditions favoring the development of a flat expanse of mycelium. Many of the fungi belonging to the Thelephoraceae form flat thalli, and it may well be that careful search among specimens belonging to this group of fungi will show that the barrage effect is a relatively common occurrence in nature. In the autumn, after the leaves have fallen, one frequently finds sheets of mycelium close to the ground under the moist leaves. It would not be surprising to find that barrages are developed between contiguous mycelial mats. They should be looked for with a view to determining whether or not the barrage effect as described by Vandendries and Brodie is a widespread or only a rare phenomenon.

The observation of the true barrage effect in a fungus under natural conditions is also of importance in being a possible indication of the heterothallism and tetrapolarity of that fungus.

Acknowledgments

It is a pleasure for the writer to acknowledge his gratitude to Professor H. S. Jackson and Dr. R. F. Cain of the Department of Botany of the University of Toronto for calling his attention to the specimen as well as to Dr. J. W. Groves for assistance in taking one of the photographs reproduced (Plate I, Fig. 3) in this article.

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AGR. RE

TEMPERATURE, WIND, HUMIDITY AND EVAPORATION IN AGRICULTURAL METEOROLOGY[1]

BY J. W. HOPKINS[2] AND MABEL F. JAMES[3]

Abstract

A statistical study has been made of evaporation and allied meteorological observations for a period of approximately 20 years at each of two stations, situated in Montana and New Mexico respectively.

The average values, over this period, for May, June, July and August of all four meteorological quantities showed a seasonal trend. At both stations July was the month of maximum average temperature, evaporation and vapor pressure. Average wind velocity was greatest at both stations in May, decreasing progressively throughout the summer.

In general the extent of evaporation was markedly influenced by wind and vapor pressure as well as by temperature. Annual variations in evaporation during a specified calendar month were found to be correlated with temperature differences, but here also there was a significant additional effect of wind and vapor pressure. Evaporation measurements should therefore supplement temperature records in agricultural meteorological studies.

Introduction

It is generally believed that the injurious effects of drought upon agricultural crop yields are intensified by high temperatures. These by increasing evaporation might be expected to accelerate drying-out of the soil and, what is probably more important, to increase the rate of transpiration and hence the water requirements of the crop.

Nevertheless statistical studies (2) of weather conditions and wheat yield in western Canada did not demonstrate any pronounced consistent effect of seasonal differences in temperature on the magnitude of the resulting crops, in spite of the fact that retardation of growth owing to deficiency of moisture frequently occurs in this area.

This result might be held to imply that in practice drought damage is largely occasioned by lack of rainfall, and is but little affected by variations in the evaporating power of the air. On the other hand, evaporation itself may be influenced by other meteorological factors to such an extent that temperature records alone fail to indicate adequately the conditions actually operative upon the crop.

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Systematic measurements of evaporation have not long been made at meteorological stations in western Canada. The Bureau of Plant Industry of the United States Department of Agriculture has, however, accumulated such observations at stations in the Great Plains area and elsewhere in the western United States over a period of approximately 20 years. Publication of these (3, 4) has made possible a statistical study of seasonal variations in evaporation from a free-water surface and their correlation with recorded observations of temperature, wind velocity, and vapor pressure, under conditions both similar and dissimilar to those prevailing in southern Saskatchewan and Alberta. In the following sections we shall analyze in this way the data for the months of May, June, July and August provided by two selected stations, one in Montana and the other in New Mexico. The investigation is purely empirical in nature, and was not undertaken with the object of adding to the existing knowledge of the laws of evaporation. Nor will it augment the number of formulas suggested for computing the extent of evaporation from the known values of meteorological factors, 11 of which formulas, being "some of the more important" will be found listed by Raman and Satakopan (5, Table I). Attention will be confined to the above mentioned aspects of variation in evaporation, its correlation with the other weather factors recorded, and the agricultural meteorological implications of the results.

Observational Data

The U.S. Department of Agriculture scheme embraced a considerable number of stations, but the observations at the majority of these did not extend over a sufficient number of years for the present purpose, or suffered from other defects, notably changes in the diameter of the evaporation pan employed, or absence of some wind or vapor pressure records. Two homogeneous series were selected for study, comprising the observations made at Moccasin, Mont. (lat. 47°15′, long. 109°58′ W. approx., alt. 4,228 ft.) and Tucumcari, N. Mex. (lat. 35°30′, long. 103°48′ W. approx., alt. 4,194 ft.). The former station is situated in longitude between Swift Current, Saskatchewan (107°40′ W., approx.) and Medicine Hat, Alberta (110°40′ W. approx.).

Tables I and II give the evaporation, temperature, wind and vapor pressure measurements for the four months May to August inclusive at Moccasin and Tucumcari, as published by Horton (3) for the period prior to 1921, and by Horton and Cole (4) for 1921–32. These authors state that the evaporation pans used were of galvanized iron, 6 ft. in diameter and 24 in. deep. The pans were buried 20 in. in the soil and were kept filled with water to soil surface level, 4 in. below the rim. Instructions required that grass should be kept cut and other obstructions removed from the vicinity so as to permit free wind movement close to the water surface. The anemometer cups were "in general" 20 in. above the rim of the pan, *i.e.*, 24 in. above the water surface.

Mean daily temperatures were obtained by averaging the readings of maximum and minimum thermometers exposed 4ft. above ground in standard

TABLE I

EVAPORATION (E_e) AND MEAN AIR TEMPERATURE (T_a), WIND MILEAGE (W) AND VAPOR PRESSURE (V) BY MONTHS AT MOCCASIN, MONT.

Year	E_e, inches				T_a, degrees F.				W, m.p.h.				V, inches Hg.			
	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.
1910	5.61	7.21	8.28	7.38	51	60	68	62	6.8	6.3	5.9	6.0	0.242	0.307	0.330	0.287
11	5.94	5.13	7.31	6.35	48	60	62	58	8.0	6.4	5.9	5.9	.271	.425	.378	.373
12	4.14	6.39	5.97	6.23	52	61	61	62	7.6	5.7	4.3	6.4	.256	.304	.336	.311
13	4.23	4.90	6.37	7.35	49	65	62	65	6.5	5.3	5.0	5.4	.238	.381	.341	.288
14	4.85	4.43	7.45	7.24	51	57	68	62	7.5	6.4	5.5	6.0	.247	.332	.372	.289
15	4.61	4.35	4.89	6.51	49	52	58	66	7.5	6.6	5.1	4.9	.229	.282	.332	.358
16	5.26	5.62	6.89	7.57	45	55	65	62	9.2	7.7	5.6	5.3	.173	.288	.382	.271
17	5.06	6.06	9.58	9.87	46	55	69	63	7.6	7.4	5.9	6.1	.187	.240	.278	.240
18	—	6.76	5.77	5.65	—	63	63	61	—	6.0	4.5	4.9	—	.311	.341	.302
19	6.37	8.57	10.00	8.74	52	64	68	67	8.0	6.9	6.6	6.3	.205	.278	.270	.253
1920	5.36	5.18	6.07	6.58	48	57	66	64	8.8	5.4	4.5	4.8	.191	.293	.365	.299
21	4.14	5.34	6.79	7.58	49	61	64	66	8.3	5.4	5.0	5.3	.223	.346	.341	.261
22	5.49	5.88	6.68	7.80	49	61	63	68	9.8	5.5	4.4	4.9	.199	.355	.336	.354
23	5.44	5.45	6.36	5.58	51	58	66	61	6.7	5.5	4.0	3.5	.221	.324	.452	.360
24	4.90	4.11	6.69	6.81	49	53	63	61	6.7	5.7	4.7	4.9	.205	.301	.315	.266
25	5.94	4.90	8.17	7.57	51	57	66	64	7.8	5.9	5.5	6.6	.211	.323	.336	.270
26	5.09	6.36	7.32	6.31	52	59	66	62	6.9	6.8	5.1	6.4	.230	.280	.358	.324
27	4.30	4.33	7.11	5.56	43	57	63	61	7.5	5.7	4.9	4.6	.197	.318	.408	.346
28	6.88	7.60	7.13	6.05	55	54	63	61	7.0	6.3	4.9	4.6	.218	.270	.402	.276
29	5.29	5.77	9.10	7.85	49	56	68	69	7.5	6.1	5.7	5.7	.204	.294	.277	.294
1930	4.94	7.33	8.28	6.82	49	59	69	67	6.8	6.9	5.3	5.2	.250	.273	.323	.353
31	5.48	6.94	8.74	7.67	52	62	67	66	7.4	6.3	5.8	5.4	.196	.307	.312	.319
32	5.28	5.19	7.69	7.72	53	58	66	65	6.7	5.0	5.2	5.7	.243	.330	.330	.318

TABLE II

Evaporation (E_e) and mean air temperature (T_a), wind mileage (W) and vapor pressure (V) by months at Tucumcari, N. Mex.

Year	E_e, inches				T_a, degrees F.				W, m.p.h.				V, inches Hg.			
	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.	May	June	July	Aug.
1913	10.35	8.72	11.83	10.14	68	71	79	79	7.3	7.2	6.6	5.2	0.292	0.410	0.400	0.419
14	7.29	9.88	8.08	8.96	64	76	76	76	7.4	7.1	3.2	3.9	.361	.470	.551	.487
15	9.91	11.24	10.02	8.41	63	74	76	73	7.1	7.1	6.1	4.3	.241	.342	.458	.441
16	11.32	12.73	11.51	8.88	67	77	80	76	7.8	7.6	5.5	5.5	.187	.302	.390	.471
17	9.48	13.72	13.41	10.02	60	76	82	75	8.2	7.3	6.4	5.4	.216	.302	.416	.450
18	12.63	12.23	12.05	11.57	68	79	80	78	10.0	6.7	6.8	7.0	.205	.377	.460	.418
19	7.78	7.54	9.21	8.56	63	70	75	77	6.7	4.7	4.9	2.9	.456	.543	.573	.561
1920	7.73	9.21	9.54	7.33	64	70	76	69	6.2	5.4	3.8	3.5	.349	.450	.533	.526
21	7.83	6.42	8.38	8.99	66	72	77	77	5.7	3.7	3.3	3.2	.352	.504	.533	.501
22	9.05	9.94	12.18	11.11	65	76	81	82	5.8	5.6	5.5	4.5	.281	.444	.435	.422
23	10.00	10.34	11.31	9.72	66	75	80	77	5.7	6.7	4.9	4.5	.274	.418	.470	.477
24	8.15	12.15	10.11	10.20	62	78	77	78	5.8	5.5	4.9	4.8	.288	.343	.481	.462
25	9.26	12.33	10.09	8.74	67	78	80	75	6.5	7.5	5.1	4.8	.324	.361	.471	.473
26	7.24	9.34	9.51	9.96	63	73	76	77	5.3	4.5	3.9	3.8	.324	.392	.450	.406
27	14.45	11.13	10.37	8.06	72	73	79	75	7.9	7.1	5.1	4.0	.181	.342	.466	.486
28	7.31	10.81	10.81	8.74	65	74	80	75	4.6	5.8	4.9	4.9	.329	.347	.449	.538
29	7.65	10.44	10.86	9.01	62	75	78	78	6.8	6.1	5.2	4.1	.330	.378	.463	.456
1930	10.24	10.66	10.01	9.30	64	76	78	79	7.9	7.0	5.2	4.2	.238	.405	.457	.458
31	7.89	10.27	9.96	8.10	61	76	78	74	5.3	4.8	4.6	3.8	.293	.425	.567	.543
32	9.07	9.47	11.41	10.03	66	73	80	78	6.1	5.0	4.9	5.1	.358	.460	.498	.477

instrument shelters. Temperature, evaporation and anemometer readings were taken at about 7 a.m. and recorded as of the previous day. The vapor pressure measurements given are the averages of the values derived from sling psychrometer readings taken at 7 a.m., 1 p.m. and 5 p.m.

Relation Between Evaporation Recorded and Temperature, Wind and Vapor Pressure Measurements

General variation and covariation

In Fig. 1 is shown for all months and seasons the amount of evaporation plotted against the mean monthly (air) temperature. The evaporation values plotted for June are 31/30 of those given in Tables I and II, thus allowing for the fact that this month comprises only 30 days. It will be seen that a high mean temperature was in general associated with above-average evaporation, and that the relation, as would be expected from physical considerations, is non-linear. Clearly however the amount of evaporation is by no means closely predictable from the mean temperature alone. The curves in Fig. 1 represent the quadratic regression equations

$$E_o = 19.27 - 0.6169T_a + 0.006669T_a^2$$

and

$$E_o = 32.00 - 0.7813T_a + 0.006509T_a^2$$

fitted to the Moccasin and Tucumcari data respectively by the method of Least Squares; but the deviations from the regression line constitute 40.2 and 71.8% respectively of the total variance of evaporation.

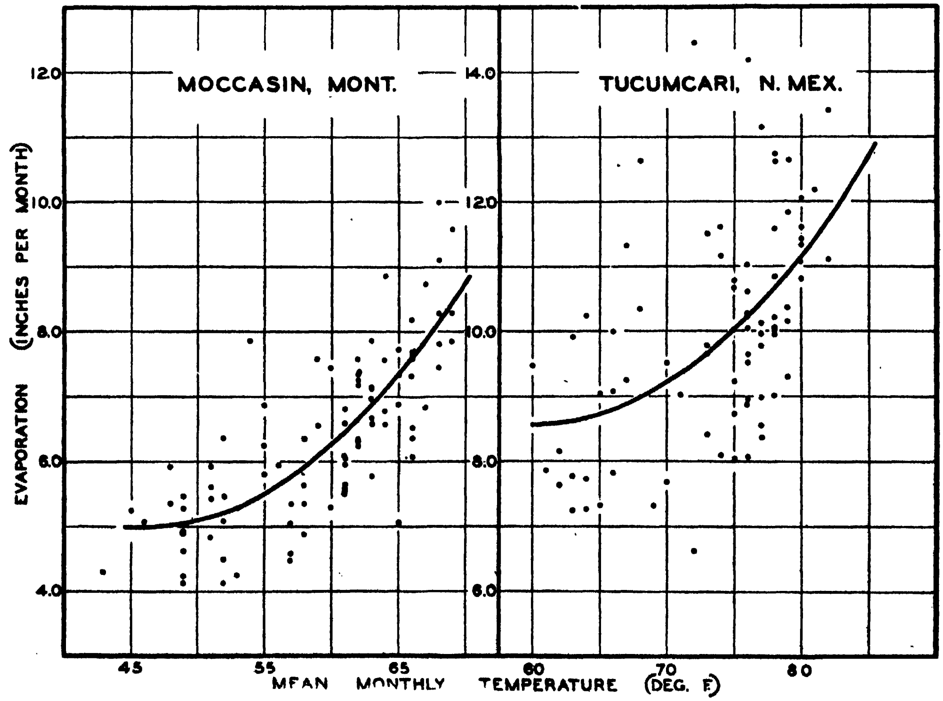

FIG. 1. *Correlation between monthly evaporation from a free-water surface and mean monthly air temperature at Moccasin, Mont., 1910–32, and Tucumcari, N. Mex., 1913–32.*

When the wind mileage and vapor pressure readings were also taken into consideration, however, these correlations were significantly enhanced. It appears that from theoretical considerations the wind effect might be expected to be proportional to the square root of the velocity rather than to the velocity itself; but the experiments of Banerji and Wadia (1) indicate that although the relation is definitely non-linear at low wind velocities, it approaches linearity over the range with which we are here concerned. In these circumstances it was felt that deviations from linearity in the relation between evaporation and average wind velocity were likely to be secondary in importance to errors introduced by the fact that the monthly average does not distinguish between a high wind blowing for a short period and one of low velocity but correspondingly extended duration. The partial regression coefficient of evaporation on mean monthly wind mileage was computed to be 0.36 in. and 0.49 in. per additional m.p.h. at Moccasin and Tucumcari respectively; the corresponding values for evaporation on vapor pressure were −0.95 and −1.06 in. per additional $\frac{1}{10}$ in. of mercury. The multiple correlation coefficient R, between evaporation and the first and second powers of the temperature, wind velocity and vapor pressure now became 0.87 for the Moccasin and 0.94 for the Tucumcari observations

These calculations suffice to indicate that there was in general a correlation between mean air temperature and monthly evaporation from a free-water surface, but that the amount of evaporation at both locations was also significantly affected by differences in vapor pressure and wind mileage. From the agricultural point of view it will be of further interest to consider the annual variation in amount of evaporation during a specified calendar month. This procedure will exclude from the estimates of variance and covariance the effects of any consistent differences between the four months in temperature, wind or vapor pressure, as well as in the temperature of the soil surrounding the evaporation pans.

Annual variation and covariation by months

The upper portion of Fig. 2 shows by columnar heights the average amount of evaporation in each of the four calendar months. Although this was in

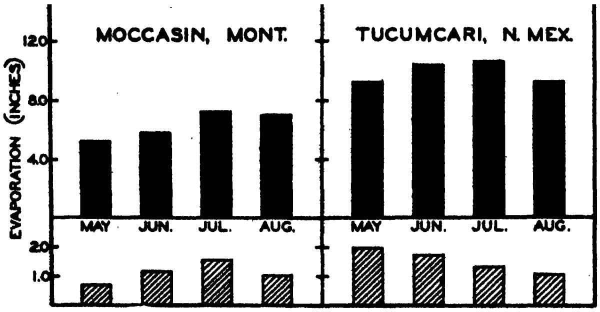

FIG. 2. *Upper portion: Average evaporation by months from a free-water surface at Moccasin, Mont., 1910–32, and Tucumcari, N. Mex., 1913–32. Below: Annual variation (standard deviation) of monthly evaporation.*

all cases higher at Tucumcari than at Moccasin, the seasonal trend is similar at both points, July having the maximum average. Annual variation in the amount of evaporation during a specified month does not, however, follow the same course at the two stations. At Moccasin July was the most variable month, but at Tucumcari annual variation was greatest in May, diminishing in the successive summer months.

Figs. 3 and 4 illustrate in a similar manner the average values and annual variation of air temperature, wind velocity and vapor pressure. They are of interest in themselves, as indicating the intra- and inter-seasonal variation of these aspects of the weather, but in particular it is to be noted that there are significant average differences between months with respect to both wind

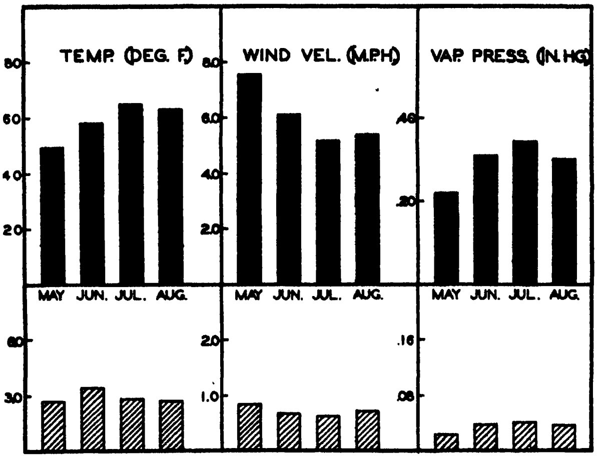

FIG. 3. *Upper portion: Average mean monthly air temperature, wind velocity and vapor pressure at Moccasin, Mont., 1910–32. Below: Annual variation (standard deviation) of mean monthly temperature, wind velocity and vapor pressure.*

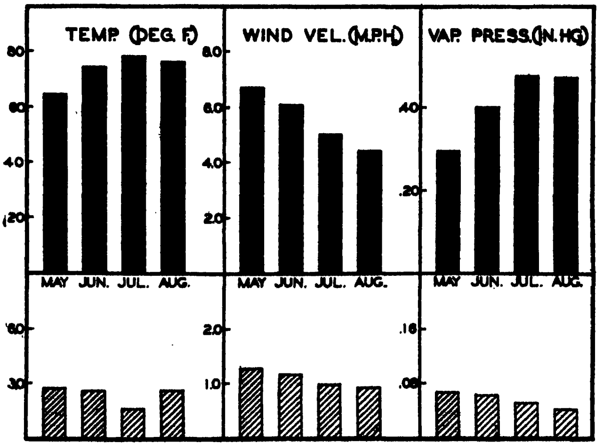

FIG. 4. *Upper portion: Average mean monthly air temperature, wind velocity and vapor pressure at Tucumcari, N. Mex., 1913–32. Below: Annual variation (standard deviation) of mean monthly temperature, wind velocity and vapor pressure.*

velocity and vapor pressure. These must have operated to reduce the direct correlation between evaporation and temperature in the observations when considered in the preceding section as a whole. At both stations July was the month of maximum average temperature, evaporation and vapor pressure. Average wind velocity was greatest in May, decreasing progressively during the summer.

Evaporation is plotted against mean monthly air temperature for the four months separately in Figs. 5 and 6. The range in temperature of a specified month from year to year is much less than that obtained when all months are thrown together, and the linear regression lines shown seem to fit reasonably adequately the data when subdivided in this way. The computed values of the regression coefficients, specifying the average increase in evaporation per unit increment of mean temperature are:

Moccasin		Tucumcari	
May,	0.114 in. per deg. F.	May,	0.487 in. per deg. F.
June,	0.145 in. per deg. F.	June,	0.511 in. per deg. F.
July,	0.352 in. per deg. F.	July,	0.584 in. per deg. F.
August,	0.193 in. per deg. F.	August,	0.301 in. per deg. F.

The regression coefficients vary somewhat from month to month at both stations. This might perhaps have been in part expected, since the average monthly mean temperature shows a seasonal trend and the effect of a given variation is greater at high than at low temperatures. It must be remembered, however, that these coefficients represent not only the direct result of temperature differences, but also any additional effect due to associated variations in wind or vapor pressure. Upon further examination it was found that the degree of association between inter-annual variations in temperature and wind and temperature and vapor pressure was not the same for all four months. When allowance was made for this situation the partial regression coefficients of evaporation on temperature, eliminating the effects of wind and vapor pressure differences, were found to be:

Moccasin		Tucumcari	
May,	0.166 ± 0.055 in. per deg. F.	May,	0.328 ± 0.054 in. per deg. F.
June,	0.257 ± 0.051 in. per deg. F.	June,	0.256 ± 0.064 in. per deg. F.
July,	0.237 ± 0.044 in. per deg. F.	July,	0.392 ± 0.062 in. per deg. F.
August,	0.171 ± 0.051 in. per deg. F.	August,	0.220 ± 0.045 in. per deg. F.

Differences between the temperature coefficients computed in this way are less pronounced, and those occurring at Moccasin cannot be regarded as statistically significant. At Tucumcari the variation was irregular, and it can be asserted only that the temperature coefficient for August is lower than that for July.

The simple correlation coefficients between evaporation and temperature were found to be:

Moccasin	Tucumcari
May, $r = +0.44$	May, $r = +0.72$
June, $r = +0.42$	June, $r = +0.71$
July, $r = +0.80$	July, $r = +0.84$
August, $r = +0.52$	August, $r = +0.77$

Evidently the recorded temperatures alone would provide a far from reliable prediction of the seasonal variation of evaporation at Moccasin. The degree of correlation is in general higher at Tucumcari, but here also there are some pronounced deviations between actual evaporation and the expectation from the temperature regression. These are in part attributable to variations in wind velocity and vapor pressure. When these variables were also taken into account, the coefficients of multiple correlation, R, between evaporation and temperature, wind velocity and vapor pressure given below were obtained:

Moccasin	Tucumcari
May, $R = 0.59$	May, $R = 0.96$
June, $R = 0.81$	June, $R = 0.95$
July, $R = 0.93$	July, $R = 0.96$
August, $R = 0.80$	August, $R = 0.92$

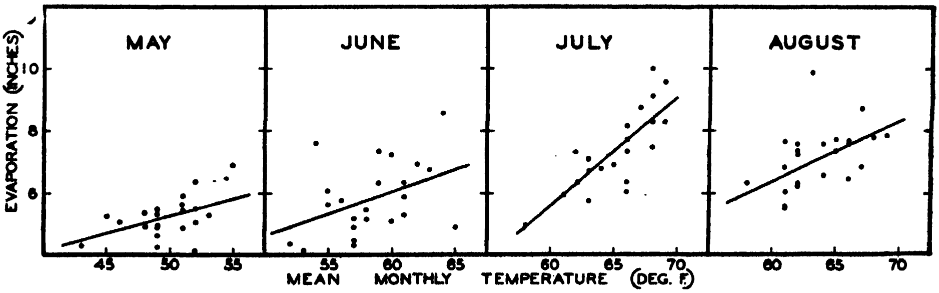

FIG. 5. *Annual variation of mean air temperature and evaporation by months at Moccasin Mont., 1910–32.*

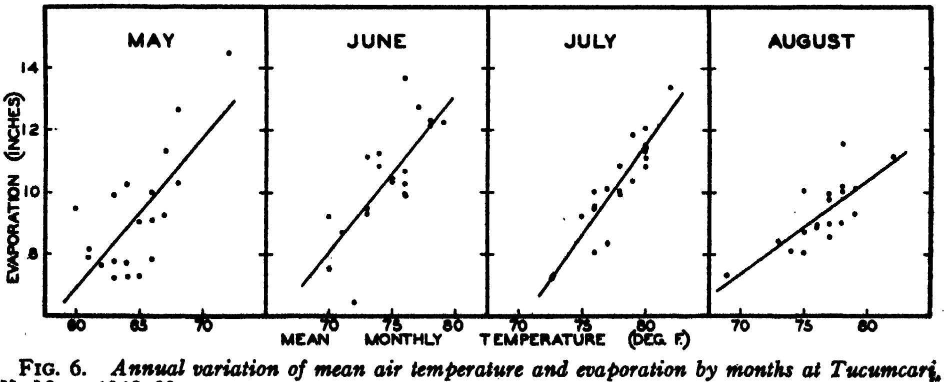

FIG. 6. *Annual variation of mean air temperature and evaporation by months at Tucumcari, N. Mex., 1913–32.*

The general degree of association at Moccasin is still lower than that at Tucumcari.

The limited number of observations results in a high standard error of the partial regression coefficients of evaporation on wind velocity and vapor pressure computed from the annual data for any one month only, since the 22 and 19 degrees of freedom at Moccasin and Tucumcari respectively can hardly be regarded as adequate for the simultaneous determination of three partial regression coefficients. It seemed reasonable to assume however that the effect of a given annual fluctuation in wind velocity or vapor pressure would be much the same for all months. Accordingly, the residual deviations of evaporation, wind velocity and vapor pressure from the temperature regressions for the separate months were combined to provide data for the calculation of an average estimate of these partial regression coefficients, the residual squares and products for June being weighted in the ratio $(31/30)^2$ and 31/30 respectively. The values found in this way were:

Moccasin	Tucumcari	
0.404 ± 0.110,	0.406 ± 0.074	in. per additional mean monthly m.p.h.
−1.13 ± 0.22,	−1.09 ± 0.15	in. per additional mean monthly $\frac{1}{10}$ in. Hg.

The agreement between the values of the coefficients computed from the observations at the two stations (which lie some 800 miles apart) is well within the standard error.

Discussion and Conclusions

Sir Napier Shaw (7) has commented on the uncertainties which may enter into comparisons of the absolute amount of evaporation owing to differences in the instruments employed or their exposure. Topographical differences are doubtless operative, and differences in altitude are also known (6) to have an effect. These, however, would be expected to influence the average amount of evaporation rather than its correlation with other meteorological factors. Nevertheless it seemed desirable to ascertain whether the moderate correlation at Moccasin might be typical of the northern plains area. For this purpose a few additional calculations were made, using the shorter 17-years' series of observations accumulated at Havre, Mont. (lat. 48°40′, long. 109°35′, approx., alt. 2505 ft.). The meteorological quantities showed an average seasonal trend similar to that already illustrated by the Moccasin data. The linear regression of evaporation on temperature was computed to be 0.210, 0.314, 0.508, and 0.330 in. per additional deg. F. mean temperature during May, June, July and August respectively. The corresponding correlation coefficients were +0.75, +0.79, +0.73 and +0.74, *i.e.*, of the same order as those previously computed for Tucumcari. It would appear therefore that the correlation at Moccasin was probably for some reason exceptionally low; but even at the other two stations, a significant proportion of the annual variation in monthly evaporation was unaccounted for by temperature differences alone.

It is not of course contended that evaporation from a free-water surface constitutes a direct measure of the coincident loss of moisture from soil either directly (especially under semi-arid conditions) or by the transpiration of plants. It does, however, provide information respecting the evaporating power of the air, which is the resultant of temperature, wind and humidity conditions; and, since the evaporating power of the air must exercise a controlling influence upon both evaporation from the soil and transpiration, evaporation data of this kind should be of definite utility in supplementing temperature records in agricultural meteorological studies. The relation between evaporation from tanks or pans of water and that from adjacent soil and vegetation would seem to merit investigation.

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STUDIES ON THE INHERITANCE OF AND THE RELATION BETWEEN KERNEL TEXTURE AND PROTEIN CONTENT IN SEVERAL SPRING WHEAT CROSSES[1]

By O. S. Aamodt[2] and J. H. Torrie[3]

Abstract

In crosses of Milturum × Selection I-28-60, F_2 populations grown from different F_1 plants were found to behave in three different ways in regard to the mode of inheritance of kernel texture. In two of these groups vitreous texture was partially dominant and governed by either one or two main factors, while in the third group starchy texture was partially dominant and governed by one main factor pair. Minor modifying factors also influence the mode of inheritance of this character in the above crosses. The heritable nature of kernel texture was also demonstrated, by correlation studies, in the crosses of Reward with Selections I-28-46, I-28-60 and I-28-62.

The inheritance of protein content in Milturum × Selection I-28-60 appeared to be controlled by polymeric factors, the exact nature of which was not determined. The lack of correlation for crude protein content between the material grown at Brooks and that grown at Fallis indicates that the expression of the mode of inheritance of protein content is readily influenced by the environment. In the other crosses studied, protein content was also shown to be inherited.

A strong positive relation was found to exist between vitreous kernel texture and high protein content for the several series of crosses studied.

Introduction

The hard red spring wheats of western Canada are noted the world over for their high milling and baking quality. In order to maintain this reputation, it is essential that the plant breeders of western Canada give the question of quality special consideration in their wheat breeding programs. At the University of Alberta, extensive studies are being carried out on several phases of this general problem. Aamodt and Torrie (2) point out that one of the most serious problems with which wheat breeders have to contend is that of securing an index of baking quality of the different hybrid lines in the early generations of a cross. The practice followed with hybrid material at the University of Alberta is to grow it at Fallis, Alberta, in the gray wooded soil area, where a differentiation in kernel texture is readily secured (1). Aamodt and Torrie (2) have shown that the kernel texture of material grown under Fallis conditions provides an excellent preliminary indication of the baking quality in the early generations of hybrid material.

As a part of the general wheat breeding program at the University of Alberta, the inheritance of kernel texture was studied in several series of crosses. The inheritance of protein content and the relation between protein content and kernel texture, under several different environments, were also studied for the same series of crosses.

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Materials and Experimental Methods

Genetic studies were made on the inheritance of, and the relation between, kernel texture and protein content in two groups of crosses. The first group consisted of crosses between Reward and Hybrid Selections I-28-46, I-28-60 and I-28-62. The second group consisted of crosses between Milturum 0.321 and Selection I-28-60. The relation between kernel texture and protein content was also studied for 100 white-seeded selections from a cross between Garnet and Selection I-28-46.

The F_1 of Reward crossed with Selections I-28-46, I-28-60 and I-28-62 was grown in the greenhouse during the winter of 1928-1929. The F_2 was grown at Edmonton (black soil) in 1929; the F_3 at Edmonton and Breton (gray soil) in 1930; and the F_4 at Edmonton and Fallis (gray soil) in 1931. The F_1 and F_2 of Milturum × Selection I-28-60 were grown at Edmonton in 1930 and 1931, respectively, and the F_3 at Edmonton (black), Fallis (gray), and Brooks (brown soil), Alberta, in 1932. One hundred white-seeded selections of Garnet × Selection I-28-46 were grown at Fallis in 1931. All the hybrid material was grown in spaced five-foot rows, one foot apart, with approximately 25 seeds per row.

The nature and composition of the black chernozem and gray podsol soils are described by Wyatt, Newton and Mather (25), and the brown soils by Wyatt and Newton (24).

Reward, C.A.N. 1621*, is an early, hard red spring wheat originating from a Marquis × Prelude cross made at the Central Experimental Farm, Ottawa. It is characterized by vitreous texture, high protein content and excellent milling and baking qualities. Garnet, C.A.N. 1316, is an early, hard red spring wheat originating from a Preston A × Riga M cross made at the Central Experimental Farm, Ottawa. It is characterized by vitreous texture, below-average protein content and fair to poor milling and baking qualities. Milturum 0.321, C.A.N. 1415, is a late, soft red spring wheat obtained from Dr. Talanov of the West Siberian Experimental Station, in 1928. It is characterized by starchy texture, low protein content and poor milling and baking qualities. Selections I-28-46, I-28-60 and I-28-62 are hard red spring wheats originating from a cross of Marquillo × Marquis-Kanred. They were introduced from the University of Minnesota, St. Paul, in 1928, and were later selected at the University of Alberta, Edmonton. They are all relatively high in protein content and possess good milling and baking qualities. At Fallis, on the gray soil, all three vary somewhat in kernel texture.

The protein content was calculated as total nitrogen × 5.7 and corrected to a 13.5% moisture basis. The material was classified for kernel texture on a scale ranging from 1 to 10; 1 represents completely starchy and 10 completely vitreous.

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* *C.A.N. = Canadian Accession Number.*

The significance of the correlation coefficients, reported herein, was judged by the method suggested by Fisher (14). A correlation was considered significant if P, the probability of exceeding the observed value through random sampling, was less than 0.05. In all instances throughout this paper the standard error is used.

Literature Review

In the early literature dealing with kernel texture there is a considerable amount of discussion on the influence of environment. Very little consideration, save in a few isolated cases, is given to the hereditary nature of kernel texture. However, more recently, considerable literature has accumulated on the mode of inheritance of this character. Owing to the importance of kernel texture in the breeding program at the University of Alberta, and in this paper, the literature on the subject is reviewed in considerable detail.

Bolley, 1905 (6) states that "yellow-berry" in wheat is determined by climatic conditions and that it is not inherited. Lyon and Kezer, 1905 (21) report that yellow-berry is produced primarily by over-ripening and the length of time exposed to the weather.

Biffen, 1905 (4) was probably the first to show clearly the heritable nature of kernel texture. In crosses between a hard Polish wheat and a soft Rivet wheat, he obtained in the F_2 a ratio of three hard to one soft for kernel texture. In a later paper, 1908 (5), in crosses between Rough Chaff (soft) and Fife (hard), he reported that the hard translucent endosperm of Fife behaved as a simple dominant to the soft opaque endosperm of Rough Chaff.

Roberts and Freeman (23), in 1908, stated that both environment and heredity play a role in determining yellow-berry. Headden, 1915 (18), studied the effect of different commercial fertilizers on yellow-berry. He concluded that the nitrogen/potassium ratio greatly influenced the production of yellow-berry; a high proportion of nitrogen favored the production of flinty kernels, while a high proportion of potassium favored yellow-berry.

Howard and Howard, 1912 (19), obtained a monogenic ratio, with an intermediate condition in the F_1, for kernel texture from crosses between soft and hard translucent wheats. Freeman, 1918 (15), studied the inheritance of kernel texture in crosses between a white macaroni wheat and (*a*) a soft red bread wheat and (*b*) Sonora 35, a soft white wheat. He proposed that two independent factor pairs, cumulative in nature, conditioned the inheritance of kernel texture in these crosses. He states that in his material there were two types of soft texture in grain; "true softness", which is only slightly affected by environmental conditions, and "yellow-berry", which is very sensitive to environmental conditions. He believed that the genetic factors governing the expression of these two types are different.

Roberts, 1919 (22), studied yellow-berry in winter wheat, from which he concluded that the operation of environmental causes of yellow-berry overshadows any differences which may be due to heredity. However, he was able to isolate pure strains of wheat which were freer from yellow-berry than others grown under identical conditions. Leith, 1919 (20), reports that the yellow-berry condition found in wheat is not heritable. However, he found considerable differences in pure lines of wheat in their tendency to produce yellow-berry. He cites the work of LeClerc and Leavitt, who also conclude that yellow-berry is caused by environmental conditions.

Bryan and Pressley, 1920 (7), crossed Turkey Red and Sonora and found the kernel texture of the F_1 to be intermediate between that of the parents. The F_2 data indicated a one-factor differential governing the inheritance of kernel texture. Harrington, 1922 (16), from the results of a series of crosses between spring wheats, concluded that the inheritance of kernel texture is governed by several factors. Hayes, 1923 (17), in a study of Marquis × Preston, obtained a correlation of $+0.407 \pm 0.059$ between the F_4 and F_5 for kernel texture. He states that, while environment plays a major role in determining kernel texture, nevertheless heredity is of importance. Engledow and Hutchinson, 1926 (13), made crosses between *T. turgidum* (soft) and *T. durum* (hard) and obtained in the F_1 completely vitreous kernels, indicating a complete dominance of the durum endosperm. They suggest that two factors govern the inheritance of kernel texture, which separately give a durum-like endosperm and jointly a true durum endosperm. Clark, Florell and Hooker, 1928 (10), studied kernel texture in Hard Federation (vitreous) × Propo (soft) wheat crosses. The F_2 and F_3 were classified into five groups for texture, as judged by the outward appearance of the kernels. The F_2 distribution approached a normal curve, with a preponderance of those possessing vitreous texture. In the F_3, they obtained indication of transgressive segregation and also true breeding intermediate lines. They concluded that kernel texture is strongly inherited and suggested that several factor pairs are involved. Bryan and Pressley, 1933 (8), crossed Baart, a vitreous wheat, and Sonora 103, a soft wheat. The F_1 plants were as soft as Sonora 103, showing a dominance of soft texture. The F_2, F_3 and F_4 showed segregation for this character and indicated a single main factor with numerous modifying factors. Aamodt, Torrie and Wilson, 1935 (3), in crosses between Reward and Milturum, report a dominance of starchy texture, and that the inheritance of kernel texture is governed by polymeric factors.

Experimental Results

Reward crosses

The F_3 of Reward crossed with each of the three Selections I-28-46, I-28-60 and I-28-62 was grown at Breton in 1930. Two hundred F_3 plants, selected at random from each cross, were grown in the F_4 at Fallis in 1931. The kernel texture index was determined for each of the F_3 plants and the average of each F_4 line. The data in Table I give the distribution, mean, and standard deviation of the hybrids and parents, for kernel texture index, for both the F_3 and F_4. The variation in kernel texture of the Reward crosses grown at Edmonton, in both 1930 and 1931 was slight. It will be noted, however, that in the case of Selection I-28-60 × Milturum a satisfactory differentiation in kernel texture was obtained in the material grown at Edmonton, in 1931 (Table III). These results can be explained on the basis that Milturum produces a starchy kernel in most environments, whereas Selections I-28-46, I-28-60 and I-28-62 require a special environment, such as that in the gray soil area, to bring out inherent differences in kernel texture. Reward is shown to be homogeneous for vitreous texture, but the great variability in kernel texture of the three Selections indicates that they are all probably heterogeneous for kernel texture. The influence of heredity on kernel texture is indicated by the close relation existing between the mean texture indices of the three Selections and those of the corresponding hybrid populations. The mean texture indices of the three Selections, for the P_3 and P_4, are: I-28-60, 8.5 and 7.6; I-28-62, 6.7 and 7.3; I-28-46, 5.8 and 5.9; while those for the corresponding crosses with Reward, in the F_3 and F_4, are: Reward × I-28-60, 8.3 and 8.2; Reward × I-28-62, 7.0 and 7.7; Reward × I-28-46, 5.8 and 7.0, respectively. These results show a direct relation between the texture indices of the parents and of the resulting hybrids, indicating that the Selections differ genetically for factors governing kernel texture. The three series of crosses show considerable variability for kernel texture. However, owing to the heterogeneous nature of the three selections used as parents, and the limited amount of material grown, a detailed genetical analysis was not possible.

TABLE I

FREQUENCY DISTRIBUTION FOR KERNEL TEXTURE OF PARENTAL LINES AND CROSSES GROWN IN THE GRAY SOIL AREA AT BRETON IN THE F_3, AND AT FALLIS IN THE F_4

Parent or Cross		Texture classes										Total number	Mean	Standard deviation
		1	2	3	4	5	6	7	8	9	10			
Reward	P_3	—	—	—	—	—	—	—	-	17	18	35	9.5 ± 0.10	0.57
	P_4	—	—	—	—	—	—	1	14	20	—	35	8.5 ± 0.10	0.60
I-28-46	P_3	—	1	1	3	1	4	1	2	2	—	15	5.8 ± 0.55	2.07
	P_4	—	—	1	3	1	4	4	2	—	—	15	5.9 ± 0.40	1.49
I-28-60	P_3	—	—	—	—	—	—	2	4	9	—	15	8.5 ± 0.18	0.68
	P_4	—	—	—	—	1	1	4	6	3	—	15	7.6 ± 0.29	1.08
I-28-62	P_3	—	1	1	—	1	3	2	5	2	—	15	6.7 ± 0.53	1.98
	P_4	—	—	—	—	1	2	6	4	2	—	15	7.3 ± 0.28	1.04
Reward × I-28-46	F_3	34	16	24	10	6	8	6	12	55	27	198	5.8 ± 0.24	3.44
	F_4	—	—	9	14	23	28	25	43	54	2	198	7.0 ± 0.13	1.84
Reward × I-28-60	F_3	—	1	7	10	11	14	9	16	66	66	200	8.3 ± 0.15	2.07
	F_4	—	—	2	4	6	14	23	30	93	28	200	8.2 ± 0.11	1.50
Reward × I-28-62	F_3	11	9	18	10	13	17	12	3	65	42	200	7.0 ± 0.21	2.92
	F_4	—	—	1	11	16	24	29	38	59	22	200	7.7 ± 0.12	1.72

Correlation coefficients were calculated between the kernel texture index of the F_3 plants and the corresponding F_4 lines, for all three series of crosses, in order to show further the heritable nature of kernel texture.

The correlations shown by the data in Table II clearly indicate that, in the crosses studied, kernel texture is a heritable character governed by definite genetic factors.

TABLE II

CORRELATIONS BETWEEN THE KERNEL TEXTURE OF THE F_3 AND F_4 PLANTS

Cross	Number	
Reward × I-28-46	198	+0.669**
Reward × I-28-60	200	+0.482**
Reward × I-28-62	200	+0.638**

** *P value exceeds the 1% point.*

Milturum crosses

Detailed genetic studies were made on the inheritance of kernel texture in the F_2 and F_3 of crosses between Selection I-28-60 and Milturum. The F_2 was grown in 1931 at Edmonton, where, owing to a moist season, a fair differentiation in kernel texture was obtained. The average texture for each F_3 line was determined at Edmonton, Fallis and Brooks in 1932. A good differential reaction for kernel texture was obtained only in the lines grown at Fallis; at Edmonton and Brooks insufficient variation occurred in kernel texture of the lines grown to make a genetical study possible.

The distribution of kernel texture within populations produced from different F_1 plants showed considerable variation both in the F_2 and F_3. The kernel texture of the grain from each plant in the F_2 populations was determined. The average for each line was calculated, and on the basis of this the populations were classified into groups. Those with a low average kernel texture were designated as starchy; those with a high average kernel texture were called vitreous and the remainder were classed as semi-vitreous. The data obtained in the F_3 were similarly treated, the progeny of each F_2

TABLE III

FREQUENCY DISTRIBUTION FOR KERNEL TEXTURE INDEX OF THE THREE TEXTURE GROUPS FROM SELECTION I-28-60 × MILTURUM AND THE PARENTAL LINES

Texture groups	Texture classes										Total number	Mean	Standard deviation
	1	2	3	4	5	6	7	8	9	10			
F_2 Edmonton, 1931													
Starchy	2	10	16	16	12	5	9	6	4	—	80	4.6 ± 0.23	2.09
Semi-vitreous	1	1	2	2	4	10	15	16	15	7	73	7.3 ± 0.22	1.91
Vitreous	1	—	—	—	1	6	21	47	34	16	126	8.2 ± 0.11	1.27
F_3 Fallis, 1932													
Starchy	14	46	39	34	13	17	21	8	2	—	194	3.9 ± 0.15	2.03
Semi-vitreous	4	10	12	14	13	26	34	29	24	2	168	6.2 ± 0.17	2.22
Vitreous	1	—	3	5	4	15	36	47	30	10	151	7.6 ± 0.12	1.47
I-28-60	—	—	—	—	1	1	2	2	4	1	11	7.9 ± 0.46	1.44
Milturum	11	—	—	—	—	—	—	—	—	—	11	1.0 ± 0.00	—

plant being regarded as a separate line. The kernel texture distributions of the lines in each group were then summed. These summations, together with their means and standard deviations, for the hybrid material grown at Edmonton in 1931 and at Fallis in 1932, and for the parental lines grown at Fallis in 1932, are given in Table III.

The data in Table III show that the distribution and the mean of the texture index of the three groups, for both the F_2 grown at Edmonton and the F_3 grown at Fallis, differ considerably. The data in Table IV show that the differences between these groups are significant in both the F_2 and F_3 and between parental lines.

TABLE IV

SIGNIFICANCE OF THE DIFFERENCE IN TEXTURE INDEX OF THE THREE TEXTURE GROUPS FROM SELECTION I-28-60 × MILTURUM AND THE PARENTAL LINES

Texture or parental groups	F_2 Edmonton		F_3 Fallis	
	Difference	Diff./S.E.	Difference	Diff./S.E.
Starchy and semi-vitreous	2.7 ± 0.32	8.4	2.3 ± 0.23	10.1
Starchy and vitreous	3.5 ± 0.25	14.2	3.7 ± 0.19	19.5
Semi-vitreous and vitreous	0.9 ± 0.25	3.4	1.4 ± 0.21	6.6
I-28-60 and Milturum	—	—	6.9 ± 0.44	15.7

Statistical significance is taken at 2 × S.E.

In the F_2 a break in the distribution occurs at class 6, while in the F_3 the break occurs at class 5. Taking these classes as arbitrary dividing points in both the F_2 and F_3, excellent fits to the expected ratios are obtained in every case, with the exception of the F_3 of the semi-vitreous group where a P value of 0.05 was obtained. The results are shown in Table V.

TABLE V

THE BREEDING BEHAVIOR OF PLANTS IN THE THREE TEXTURE GROUPS FROM SELECTION I-28-60 × MILTURUM

Generation and texture group	Class	Observed	Calculated	χ^2	P lies between
F_2 starchy	starchy	61	60	0.07	0.80 and 0.70
	vitreous	19	20		
F_2 semi-vitreous	starchy	20	18.3	0.21	0.70 and 0.50
	vitreous	53	54.7		
F_2 vitreous	starchy	7	7.9	0.11	0.80 and 0.70
	vitreous	119	118.1		
F_3 starchy	starchy	146	145.5	0.01	0.95 and 0.90
	vitreous	48	48.5		
F_3 semi-vitreous	starchy	53	42	3.84	0.05
	vitreous	115	126		
F_3 vitreous	starchy	12	9.4	0.77	0.50 and 0.30
	vitreous	139	141.6		

The data indicate that one or both of the parents are heterogeneous for kernel texture. Phenotypically Milturum is homogeneous for starchy endosperm. In 1932 all the parental lines of Milturum when grown at Fallis had a texture index of 1, while Selection I-28-60 had a range from 5 to 10, which indicates that Selection I-28-60 may be heterogeneous for kernel texture. One very interesting aspect of this study is that in certain F_2 populations starchy texture is partially dominant by one main factor and in others vitreous texture is partially dominant by either one or two main factors. In the F_3, grown at Brooks and Edmonton, there was not sufficient variation in kernel texture to allow a detailed study of this character. However, it was noticed that invariably the starchy texture groups had a much higher proportion of starchy plants at both Edmonton and Brooks than either the semi-vitreous or vitreous texture groups. A review of the literature shows that in some cases vitreous texture had been found dominant and in other cases starchy or soft texture was dominant. The only possible explanation for the behavior of the material in these crosses is that Selection I-28-60 is heterogeneous for factors governing kernel texture. In the vitreous texture group there must be two main factors governing texture, and in the semi-vitreous group one main factor, and in both cases a partial dominance of vitreous texture, while in the starchy texture group, starchy texture is partially dominant by a single factor.

The inheritance of kernel texture is also probably influenced by a number of modifying factors as well as the main factors indicated above. Correlation coefficients were calculated between the kernel textures of the three texture groups, grown in the F_2 at Edmonton and in the F_3 at Fallis. These data are shown in Table VI.

TABLE VI

INTER-GENERATION CORRELATIONS FOR THE THREE TEXTURE GROUPS OF PLANTS FROM SELECTION I-28-60 × MILTURUM

Variables correlated		Total number	r
Vitreous group	F_2 and F_3	123	+0.404**
Semi-vitreous group	F_2 and F_3	63	+0.706**
Starchy group	F_2 and F_3	80	+0.885**

** *P value exceeds the 1% point.*

Protein Content

Literature review

Protein content is one of the most important indications of quality in wheat. With the exception of the papers of Clark and his associates, there is a dearth of literature on the mode of inheritance of protein content in wheat.

Biffen, 1908 (5), working with crosses between a Polish wheat high in nitrogen content and Rivet wheat, low in nitrogen content, obtained segregation in the F_2 for this character. He states that high nitrogen content was associated with hard translucent kernels. Clark, 1926 (9), obtained transgressive segregation for protein content beyond the upper extremes of both parents in the F_4 of reciprocal crosses of Kota × Hard Federation. Clark and Hooker, 1926 (11), with reciprocal crosses of Marquis and Hard Federation, found that the protein content for the F_2 and F_3 of the hybrids was intermediate between that of the parents, and that there was no indication of transgressive segregation. They state that protein content is inherited in intermediate degrees between the two parents by means of multiple factors. Correlations between the protein content of the F_2 and the F_3 were not significant, which illustrates how readily this character is influenced by the environment. Clark, Florell and Hooker, 1928 (10), in a series of crosses between Bobs, Hard Federation and Propo, found a tendency for low protein content to be dominant. Correlations between the F_2 and F_3 were not significant. Clark and Quisenberry, 1929 (12), found that the F_2 and the F_3 of a Marquis × Kota cross were intermediate between, and more variable than, either parent. Several F_3 strains had a lower protein content than Marquis the lower parent, indicating transgressive segregation. Correlations between the F_2 and F_3 protein content were highly significant.

Experimental results

Reward crosses. In the crosses of Reward with Selections I-28-46, I-28-60 and I-28-62, the protein content was determined on 20 lines of each cross grown in the F_3 at Edmonton and Breton, and in the F_4 at Edmonton and Fallis, but not on the F_3 of Reward × Selection I-28-46 at Edmonton. The F_3 lines chosen for the protein determinations were selected on the basis of their F_2 behavior for texture. Ten lines of each cross with a starchy texture and ten with a vitreous texture were used.

The correlation coefficients obtained for the various comparisons of protein content in the crosses of Reward with Selections I-28-46, I-28-60 and I-28-62 are given in Table VII. In each of the correlations a population of 20 was used.

TABLE VII

INTER-GENERATION AND INTER-STATION CORRELATIONS FOR PROTEIN CONTENT OF WHEAT

Crosses and association	*r*
Reward × I-28-46, F_3 Breton and F_4 Fallis	0.510*
Reward × I-28-60, F_3 Breton and F_4 Fallis	0.848**
Reward × I-28-62, F_3 Breton and F_4 Fallis	0.766**
Reward × I-28-60, F_3 and F_4 Edmonton	0.375
Reward × I-28-62, F_3 and F_4 Edmonton	0.441*
Reward × I-28-46, F_3 Edmonton and Breton	0.510*
Reward × I-28-60, F_3 Edmonton and Breton	0.257
Reward × I-28-60, F_4 Edmonton and Fallis	0.701**
Reward × I-28-62, F_3 Edmonton and Breton	0.631**
Reward × I-28-62, F_4 Edmonton and Fallis	0.620**

* *P value exceeds the 5% point.* ** *P value exceeds the 1% point.*

TABLE VIII

FREQUENCY DISTRIBUTIONS FOR PROTEIN CONTENT OF WHEAT GROWN AT FALLIS, EDMONTON AND BROOKS, IN 1932

	Classes for protein content in per cent																	Number	Mean	Standard deviation
	9.8	10.3	10.8	11.3	11.8	12.3	12.8	13.3	13.8	14.3	14.8	15.3	15.8	16.3	16.8	17.3	17.8			
Fallis (gray soil)																				
Milturum	—	—	—	—	—	1	2	3	—	1	2	—	—	—	—	—	—	9	13.5±0.47	1.41
Selection I-28-60	—	—	—	—	—	—	—	1	—	3	3	2	—	—	—	—	—	9	14.6±0.19	0.57
F_3	3	12	15	12	19	11	15	11	14	11	5	4	—	—	—	—	—	132	12.4±0.13	1.45
Edmonton (black soil)																				
Milturum	—	—	—	—	—	1	—	1	1	—	—	—	—	—	—	—	—	3	13.1	—
Selection I-28-60	—	—	—	—	—	—	—	—	—	—	—	1	1	1	—	—	—	3	15.8	—
F_3	—	—	—	—	—	—	—	—	—	8	5	11	4	7	2	5	—	42	15.4±0.15	0.96
Brooks (brown soil)																				
Milturum	—	—	—	—	—	—	—	—	—	—	—	1	—	1	1	—	—	3	16.2	—
Selection I-28-60	—	—	—	—	—	—	—	—	—	—	—	—	1	2	—	—	—	3	16.2	—
F_3	—	—	—	—	—	—	—	—	—	—	—	4	6	13	15	3	1	42	16.4±0.09	0.57

For all but two comparisons, the correlations are significant. The correlations of Reward × Selection I-28-60 both between the F_3 and F_4 grown at Edmonton and between the F_3 grown at Edmonton and Breton were positive but not significant. These data clearly demonstrate that protein content is a heritable character.

Milturum crosses. The protein content was determined on 42 F_3 lines from Milturum × Selection I-28-60 crosses, grown at Brooks and Edmonton, and 132 F_3 lines grown at Fallis, in 1932. In Table VIII the data give the distribution, mean and standard deviation for the F_3 lines and parents of Milturum × Selection I-28-60 grown at the three different stations.

The protein determinations of the grain from parental lines, grown at Edmonton and Brooks, were too few in number for statistical analysis of the data. The data may be used, however, to show the general trend in protein content of the parental lines. When grown at Edmonton, there was a difference of 2.7% in the average protein content of the parents, indicating an inherent tendency for Selection I-28-60 to be higher in protein content than Milturum. When grown at Brooks, however, both parents had an average protein content of 16.2%. The fact that no difference occurred in the protein content of the parental lines grown at Brooks indicates that the dry soils, and consequent low yields obtained in this area, afford a poor differentiation for the study of inherent differences in protein content. When grown at Fallis, a difference of 1.1 ± 0.51% was obtained between Selection I-28-60 and Milturum, which indicates that under Fallis conditions Selection I-28-60 has the same inherent capacity for the production of protein as Milturum. The F_3 grown at Fallis showed considerable transgressive segregation for protein content beyond that of the low parent, Milturum. No lines with a higher protein content than Selection I-28-60 were obtained. In the F_3 lines grown at both Edmonton and Brooks indications of transgressive segregation were obtained. However, owing to the small number of parental lines studied, these indications cannot be considered significant.

The average protein content of the F_3 lines grown at Fallis, Edmonton and Brooks of 12.4 ± 0.13, 15.4 ± 0.15, and 16.4 ± 0.09, respectively, are all significantly different. This clearly illustrates the great influence which different environmental conditions, such as climate and soil type, can have upon the protein content of the same material. The results show that the genetic factors governing this character can best be determined in an environment which gives a different phenotypic expression to each of the genotypic combinations. The distribution in Table VIII indicates that polymeric factors govern the mode of inheritance of protein content in this cross.

The data in Table IX give the correlation coefficients for protein content between the F_3 lines grown at Edmonton, Brooks and Fallis. The correlation between Edmonton and Fallis is highly significant, but between Edmonton and Brooks, and Brooks and Fallis, the correlations are not significant. These results demonstrate further that at Brooks, on the dry brown soils, there is little differentiation between the lines because the environment dominates to such an extent that the protein content of all varieties tends to reach a common maximum. At Edmonton on the black soils the influence of environment is less than at Brooks, and certain lines show greater variations in protein content from the average. At Fallis, on the gray soil, heredity becomes a more dominating factor in determining the phenotype, and there is a consistent wide difference in protein content between different lines. Only those lines which have a strong hereditary tendency to produce high protein will be above the general average.

TABLE IX

INTER-STATION CORRELATIONS FOR PROTEIN CONTENT OF MILTURUM × SELECTION I-28-60 F_3 LINES

Variables correlated	Total number	
Edmonton and Fallis	42	+0.509**
Edmonton and Brooks	42	+0.289
Brooks and Fallis	42	−0.160

** *P value exceeds the 1% point.*

The Relation of Kernel Texture to Protein Content

Literature review

The literature on the relation between kernel texture and protein content in varietal studies reviewed by Aamodt and Torrie (2) shows that certain workers reported associations between kernel texture and protein content, while other workers found no association. The relation between these two characters in genetic studies has been studied by Clark and his co-workers. Clark, 1926 (9), with Hard Federation × Propo crosses grown at Davis, California, found a high positive correlation between texture and protein content in the F_3. He states, however, that high protein content is not necessarily associated with hard texture, which is indicated by both the lack of significant correlations between the F_2 and the F_3 for protein content and the partial dominance of low protein content and vitreous texture in the F_2. Clark, Florell and Hooker, 1928 (10), in crosses between Bobs, Hard Federation and Propo wheats, report that there is a slight tendency toward increased protein content with the increase in vitreousness of the kernel texture.

Experimental results

The relations existing between the two characters, kernel texture and protein content, were studied in three groups of crosses. These are: Reward crossed with Selections I-28-46, I-28-60 and I-28-62, in both the F_3 grown at Breton and the F_4 grown at Fallis, the F_3 of Milturum × Selection I-28-60, grown at both Edmonton and Fallis, and 100 white seeded F_4 lines of Garnet × Selection I-28-46 grown at Fallis.

Reward crosses. The data in Table X show that in every case the difference in protein content between the vitreous and starchy textured lines is statistically significant, with the one exception of the F_3 of Reward × Selection I-28-62, grown at Edmonton. The difference between the vitreous and

TABLE X

FREQUENCY DISTRIBUTION FOR PROTEIN CONTENT, OF THE VITREOUS AND STARCHY TEXTURE F_3 AND F_4 LINES GROWN ON BOTH THE GRAY AND BLACK SOILS

Material		Classes for protein content in per cent													Mean	Standard deviation
		8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5		
Reward × I-28-46 F_3 Breton	V[1]	–	–	2	2	3	3	–	–	–	–	–	–	–	12.2±0.37	1.10
	S[2]	–	5	4	1	–	–	–	–	–	–	–	–	–	10.1±0.22	0.66
F_4 Fallis	V	–	–	–	5	4	1	–	–	–	–	–	–	–	12.1±0.22	0.66
	S	–	–	3	7	–	–	–	–	–	–	–	–	–	11.2±0.15	0.46
F_4 Edmonton	V	–	–	–	–	–	–	–	–	5	5	–	–	–	17.0±0.21	0.63
	S	–	–	–	–	–	–	3	4	3	–	–	–	–	15.5±0.26	0.77
Reward × I-28-60 F_3 Breton	V	–	–	–	1	–	2	2	3	2	–	–	–	–	14.7±0.49	1.48
	S	–	–	2	5	1	2	–	–	–	–	–	–	–	11.8±0.33	1.00
F_3 Edmonton	V	–	–	–	–	–	–	–	–	–	5	5	–	–	18.0±0.21	0.63
	S	–	–	–	–	–	–	–	–	3	6	1	–	–	17.3±0.20	0.60
F_4 Fallis	V		–	–	–	1	6	3	–	–	–	–	–	–	13.7±0.20	0.60
	S	–	–	4	3	2	1	–	–	–	–	–	–	–	11.5±0.33	1.00
F_4 Edmonton	V	–	–	–	–	–	–	–	–	5	4	1	–	–	17.1±0.22	0.66
	S	–	–	–	–	–	–	–	6	4	–	–	–	–	15.9±0.19	0.58
Reward × I-28-62 F_3 Breton	V	–	–	–	–	1	4	3	2	–	–	–	–	–	14.1±0.32	0.97
	S	1	1	6	2	–	–	–	–	–	–	–	–	–	10.4±0.28	0.84
F_3 Edmonton	V	–	–	–	–	–	–	–	–	–	5	4	1	–	18.1±0.22	0.66
	S	–	–	–	–	–	–	–	–	1	7	2	–	–	17.6±0.18	0.54
F_4 Fallis	V	–	–	–	1	4	2	1	1	1	–	–	–	–	13.5±0.49	1.48
	S	–	2	1	5	2	–	–	–	–	–	–	–	–	10.9±0.43	1.28
F_4 Edmonton	V	–	–	–	–	–	–	–	–	3	4	1	1	1	17.8±0.43	1.27
	S	–	–	–	–	–	–	1	3	3	3	–	–	–	16.3±0.33	0.98

[1] *V = Vitreous.* [2] *S = Starchy.*

starchy textured lines, in this case, is slightly under twice the standard error of the difference. It is interesting to note that, in spite of the fact that the difference in kernel texture of the vitreous and starchy textured lines was masked by the environment, when grown at Edmonton, the difference in protein content of the two groups of lines was statistically significant for all series of crosses in both the F_2 and F_3, with the one exception noted above. This clearly demonstrates the necessity of growing the material in a differential environment, in order to show differences in quality by kernel texture. These results show the close relation which exists between protein content and the kernel texture, as determined on material grown in the gray soil area. The great influence of environment on protein content is illustrated by the differences in protein content, ranging from three to six per cent, between samples of the same material grown on the two different soil types.

The mean protein content and kernel texture of the parents used in the above crosses are shown by the data in Table XI. Since the protein content was determined on only three samples of each parent, for any one mean, standard deviations were not calculated to show the variability. The variation in protein percentage, in all cases, was small, rarely exceeding one per cent.

TABLE XI

MEAN PROTEIN CONTENT AND KERNEL TEXTURE OF PARENTAL LINES IN 1931

Parent	P_3				P_4			
	Breton		Edmonton		Fallis		Edmonton	
	Protein, %	Kernel texture	Protein, %	Kernel texture	Protein, %	Kernel texture	Protein, %	Kernel texture
Reward	12.5	9	17.8	10	13.1	9	17.7	10
I-28-46	11.2	7	15.2	10	11.4	7	—	10
I-28-60	11.7	8	16.3	10	11.0	7	15.4	10
I-28-62	10.7	7	15.9	10	11.0	6	18.0	10

The data in Table XII give the correlation coefficients between kernel texture and protein content of Reward crossed with Selections I-28-46, I-28-60 and I-28-62 for both the F_3 and F_4 generations. The correlations are based on populations of 20 in each case.

These correlations are all highly significant and further indicate that, when the material is grown on the gray soils, there exists a very strong positive correlation between kernel texture and protein content.

TABLE XII

CORRELATIONS BETWEEN KERNEL TEXTURE AND PROTEIN CONTENT

Cross and station	r
Reward × I-28-46, F_3 Breton	+0.841**
Reward × I-28-46, F_4 Fallis	+0.548*
Reward × I-28-60, F_3 Breton	+0.786**
Reward × I-28-60, F_4 Fallis	+0.849**
Reward × I-28-62, F_3 Breton	+0.921**
Reward × I-28-62, F_4 Fallis	+0.899**

* *P value exceeds the 5% point.*
** *P value exceeds the 1% point.*

Milturum crosses. It was previously shown that, in the crosses of Milturum with Selection I-28-60, there were three distinct groups of F_3 populations for the mode of inheritance of kernel texture. In Table XIII will be found the correlation coefficients for each of the three texture groups grown at Fallis and for the total population, when grown at both Edmonton and Fallis, between kernel texture and protein content.

TABLE XIII

CORRELATIONS BETWEEN PROTEIN CONTENT AND KERNEL TEXTURE FOR THE F_3 OF MILTURUM × SELECTION I-28-60

Variables correlated	No. of samples	r
Texture and protein, vitreous texture group at Fallis	30	+0.668*
Texture and protein, semi-vitreous texture group at Fallis	39	+0.473*
Texture and protein, starchy group at Fallis	63	+0.551*
Texture and protein, total population at Fallis	132	+0.503*
Texture and protein, total population at Edmonton	42	−0.027

* *P value exceeds the 1% point.*

The data in Table XIII show that the correlations between these two characters are very significant for the hybrids grown at Fallis, but not for those grown at Edmonton. The environmental conditions at Edmonton during the growing season of 1932 were not favorable for securing a good differentiation in kernel texture. As a result very little variation was obtained for the kernel texture of the lines grown, and this may account, to a large degree, for the lack of a significant correlation between protein content and kernel texture of the hybrids grown at Edmonton in the year 1932. It was thought that since kernel texture of the hybrids grown at Fallis was so strongly correlated with protein content, there would be a wide difference in the average protein content of the three texture groups. This was not found to be the case. The average protein content was 12.2 ± 0.25, 12.5 ± 0.40 and 12.7 ± 0.37 for the starchy, semi-vitreous and vitreous textured groups, respectively. The differences between the average protein contents of the three groups are not significant. The reason for this apparent paradox is shown by the data in Table XIV.

TABLE XIV

RELATIONS EXISTING BETWEEN THE TEXTURE INDEX AND PROTEIN CONTENT, FOR THE F_3 OF MILTURUM × I-28-60, GROWN AT FALLIS IN 1932

Range of texture index	Average percentage of protein		
	Starchy texture group	Semi-vitreous texture group	Vitreous texture group
1–5	11.9±0.33	11.9±0.50	11.0±0.57
6–10	13.3±0.83	12.9±0.56	13.2±0.51
Ave. 1–10	12.2±0.25	12.5±0.40	12.7±0.37

The data in Table XIV show that the more starchy textured lines of the starchy texture group had a higher protein content than the starchy textured lines of the vitreous texture group; the same relation also held for the more vitreous textured lines of these groups. These differences were sufficient to balance the expected significant differences between the groups in average protein content.

Garnet crosses. A number of F_4 white-seeded segregates from a Garnet × Selection I-28-46 cross were grown at Fallis in 1931. The selections were classified into four categories for kernel texture. These categories were: vitreous, semi-vitreous, semi-starchy and starchy. The protein content was determined on 100 individual plants, 25 from each category. The mean protein content for each of the four categories is shown by the data in Table XV.

These data show that there is a progressive decrease in the mean protein content of the samples, with an increase in starchiness. The test of independence between kernel texture and protein content for the above material gave a P value of less than 0.01. This clearly demonstrates the high degree of association between these two characters for the material when grown under Fallis conditions.

TABLE XV

THE MEAN PROTEIN CONTENT OF F_4 WHITE SEEDED SEGREGATES FROM A CROSS OF GARNET × I-28-46, GROWN AT FALLIS, IN 1931

Category	Mean protein content
Vitreous	13.0 ± 0.25
Semi-vitreous	11.0 ± 0.27
Semi-starchy	9.4 ± 0.44
Starchy	9.2 ± 0.16

Discussion

The importance of some of the problems connected with securing an indication of the quality of hybrid material in early generations has been discussed by Aamodt and Torrie (2). They have suggested the growing of the material on the gray soils in an environment such as that at Fallis, Alberta, where a good differentiation in kernel texture is obtained. They show by correlation studies that this character is of considerable value as a means of discarding hybrid lines of low quality. In conjunction with the above studies, the present experiments were carried out to determine the heritable nature of, and the relation between, protein content under different environments for several series of crosses.

The heritable nature of kernel texture, shown in the crosses studied, is in agreement with the conclusions of other workers studying hybrid material (3–5, 7, 8, 10, 13, 15–17). The important effect of environment on the expression of kernel texture, as shown by the results obtained at Edmonton and Brooks, is in agreement with the statements of Roberts and Freeman (23) and Hayes (17). This effect clearly indicates the necessity of growing the material under differential environment, such as at Fallis, that will permit the expression of the genetic factors governing kernel texture.

Protein content is also a character of considerable value in preliminary determinations of baking quality. The polymeric factor hypothesis for the mode of inheritance of this character is in agreement with the results obtained by Clark and Hooker (11). The great influence that environmental factors, such as climate and soil type, have on protein content agrees with the results reported by Clark and his co-workers (10, 12). The results show that the genetic factors governing this character can best be determined in an environment which gives the maximum phenotypic expression for each genotype.

The relation between kernel texture and protein content is highly significant for all the crosses studied. In the case of the crosses of Reward with Selections I-28-46, I-28-60 and I-28-62, where differences in kernel texture were masked at Edmonton by the environment, the differences in protein content of the starchy and vitreous groups were significant, in both the F_2 and F_3 in all cases save one.

The heritable nature of the two characters of kernel texture and protein content, and their relations with loaf volume and partial baking score, indicate that they are of considerable value as indices of baking quality. The simplicity of the kernel texture method, as shown by Aamodt and Torrie (2), when tested on material grown at Fallis, and its much closer relation with baking quality determined on material grown at Edmonton, than that of protein content, indicates that kernel texture is the better index for baking quality. The importance of growing the material to be tested under an environment which will give a differential reaction for kernel texture every year cannot be overemphasized.

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STUDIES ON BROWNING ROOT ROT OF CEREALS[1]

III. PHOSPHORUS-NITROGEN RELATIONS OF INFESTED FIELDS
IV. EFFECTS OF FERTILIZER AMENDMENTS
V. PRELIMINARY PLANT ANALYSES

By T. C. Vanterpool[2]

Abstract

These papers deal with the elucidation of the factors which predispose wheat seedlings following summerfallow to *Pythium* root rot in certain parts of Saskatchewan. The chemical soil analyses on 66 sample pairs reveal that available phosphorus in the soil is lower and nitrate nitrogen higher in browning patches than in normal patches. Greenhouse experiments and field observations show that phosphate amendments at the time of seeding, and farmyard manuring, permit the development of normal plants in browning soil; cereal straw applications inhibit the disease to some extent, while inorganic nitrogenous fertilizers usually have no effect or occasionally may prove detrimental. Preliminary plant analyses indicate that the total phosphorus in diseased plants is lower than in healthy ones, and that there is relatively more water-soluble nitrogen and relatively less protein nitrogen in diseased than in healthy plants.

The soil and crop-culture factors, especially the practice of bare summerfallowing, which may influence the available phosphorus-nitrate nitrôgen ratio in the soil, are discussed in relation to disease incidence. It appears that any crop practice which tends to increase the available phosphorus-nitrate ratio in the soil inhibits the disease, and conversely. This is used as a basis for control recommendations.

Attention is drawn to the striking similarity of the factors affecting the severity of *Pythium* root rot of wheat and *Pythium* root rot of sugar cane in Hawaii.

It is considered that an improper balance of available phosphorus and nitrate nitrogen in the soil leads to an unbalanced metabolism of the wheat seedlings at a critical stage in their development, thereby predisposing them to fungal attack. The effect of the unbalanced nutrients on the parasitic vigor of the pathogen still requires elucidation.

III. PHOSPHORUS-NITROGEN RELATIONS OF INFESTED FIELDS

The parasitic nature of browning root rot was demonstrated in a previous communication (46) and its severity on the wheat crop following summerfallow was pointed out. It is caused primarily by species of *Pythium* (of which *P. arrhenomanes* Drechs. is probably the chief) which attack the root tips and fine laterals during the first few weeks of growth and by the fifth or sixth week produce a yellowish-brown discoloration of the outer leaves and a retardation of growth. The shift in location from one year to another of severely diseased zones in central and north-central Saskatchewan demonstrates clearly the close correlation of the disease with environmental factors and that these are of more importance than crop sequence in determining its severity. Thus since the browning root-rot epidemic of 1928, the yearly magnitude of losses has varied but little over the province as a whole. These papers present a general consideration of the problem together with data on the relation of soil fertility and plant nutrition to disease incidence.

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General Considerations

Evidence from our own findings and observations, and from the work of others, made it reasonable to postulate that unbalanced soil nutrients possibly increased the liability of the seedlings to attack from the soil-borne parasites at a critical stage in their development. A general consideration of the factors likely to be involved, as they appertain to prairie conditions, will place the problem in its true perspective.

Nitrogen is probably the soil nutrient most influenced by summerfallowing. The work of Wyatt and co-workers (49) and of Newton (30) showed that nitrate nitrogen in prairie soils is highest during May and early June on land which was summerfallowed the previous season. Richardson and Fricke (37) reported that in Australia there is a negative response of wheat after bare fallow to nitrogen applications owing to the accumulation of nitrate sufficient for or in excess of the heavy demands of the fallow crop. Ellis (14) also found that on the open prairie soils of Manitoba no positive response of wheat on fallow was obtained from sodium nitrate or ammonium sulphate. The thorough cultivation increases the activities of the nitrifying micro-organisms and thus ensures a greater accumulation of nitrate. Bedford (4) considered that the potentiality of semi-arid prairie soils for rapid nitrification can best be explained by estimating the bacterial numbers not on the soil aggregate, but on the organic matter as a unit. Land bearing a second crop of wheat is relatively low in nitrate (30, 37, 49), which has been removed by the previous crop and by micro-organisms in the decomposition of the crop residue. Richardson and Fricke (37) also found that in Australia, wheat on stubble responded to nitrogenous fertilizers. Recent work on the fertilization of the Canadian prairies is in general agreement with the findings of the Australian workers. In this connection it should be noted that *Pythium* root rot is most severe in June on wheat on well worked fallow, and is seldom of any importance on the stubble crop (45, 46). In Hawaii, Carpenter (6, 7) put forward the contention that an excess of nitrogenous fertilizers rendered the sugar cane more susceptible to root rot by the same group of fungi which causes browning root rot of cereals. Also, that the damage was considerably lessened when sugar cane fibre, almost entirely cellulose, was previously incorporated with the soil. That is, susceptibility of cane roots to attack of *Pythium* might be acquired or lost depending on the quality or balance of nutrients. This doubtless involves the complex questions of the carbohydrate-nitrogen ratio and the biological activities of soil organisms.

There are numerous references in plant disease literature, many of them, it must be confessed, without detailed supporting data, which indicate generally that excess nitrogenous fertilizers render plants more susceptible to disease, providing phosphate or potash or both are present in limited amounts. A general reference on these points need only be made to the literature compilations of Wilson (48, pp. 164-165). It is not likely that there is a deficiency of potash in the open prairie soils of Saskatchewan (22), but in many districts there is a limited amount of available phosphorus as shown by chemical determinations, and the marked response of wheat on fallow to phosphate fertilization (29). Results of a similar nature with regard to phosphorus have been obtained in the adjoining province of Alberta by Wyatt and Ward (50), and in Manitoba by Ellis (14). Nygard (31) reported a positive response of spring wheat to phosphates on many soils in Montana. The suggestion arose that a low content of available phosphate may in some way be contributory to the *Pythium* root rot. It is an interesting fact that the amount of available phosphorus in a given prairie soil appears to be fairly constant throughout the year, as indicated by Mitchell (29). Our findings on a small number of fields are in agreement with this (Table VIII). However, Emmert (15), Meyer (28) and Sturgis (42) have indicated that a correlation might exist between available phosphorus and organic matter in the soil, the state of decomposition of the latter being the chief determining factor. Richardson and Fricke (37) have pointed out that it is the customary practice in wheat-growing sections in Australia to apply phosphate to wheat following fallow. The increasing use of phosphate fertilizers on the Canadian prairies during the last few years indicates that the same will soon be true for wheat culture in the Canadian West.

The tendency for phosphorus to decrease soil nitrification, as indicated by several workers, might have some bearing on the problem. According to Wyatt and Ward (50) the application of phosphates to certain soils in Alberta tended to decrease nitrification. Ivanovskaia (21) found that in alkaline soil highly resistant to oxidation, water-soluble phosphorus pentoxide decreased when the nitrate content was high and increased when it was low.

There is also the possibility that there exists an antagonistic or a reciprocal relation between phosphate and nitrate availability or accumulation in the soil and their absorption by the plant (19, 20, 24, 25). Hoagland (19) has pointed out that, "Under appropriate conditions of solution technique, in experiments with plants such as wheat or barley, it may be shown that there exists a reciprocal relation between nitrate and phosphate. By decreasing the concentration of phosphate in the culture medium at certain stages of growth, the plant may be caused to accumulate more nitrogen. Conversely, by decreasing the concentration of nitrate, plants may sometimes be caused to accumulate a very high content of phosphate". In general, the findings of MacGillivray (24), McCool and Cook (25), Huelsen (20), and Chapman (9) support the same views. McCool and Weldon (26), Davidson (12), Beaumont (3) and Chapman (9), obtained indications that nitrate influenced the availability of phosphorus in the soil. Breazeale (5), however, could find no reciprocal relation between nitrogen and phosphorus in solution cultures. Smith and co-workers (41) reported that sodium nitrate applications increased the amount of available phosphorus in the soil from 12.5 p.p.m. to 18.6 p.p.m. in a Carrington loam in Iowa. The investigational results of Pearsall and Ewing (33), Cook (10), Emmert (15), Pettinger (34), Sessions and Shive (39), and Chapman (9), are in general agreement with this view. Thomas (43), citing from the work of Sekera (38), in summarizing the current view stated that "All experimental evidence appears to indicate that the plant, especially in the early stages of growth up to blooming, absorbs the nutrient ions in approximately the same proportion that these ions are present in the nutritive medium". It would appear then generally that, under conditions of unbalanced phosphate and nitrate in the nutrient medium, the anions tend to have an antagonistic or a reciprocal effect on the rate of absorption of each other. Under more balanced conditions, especially during the first few weeks of growth, the phosphate and nitrate are found in the cell sap in relatively the same proportions as in the external solution.

The work of Cooke (11) on the relation of high phosphate-fixing capacity of Hawaiian soils to *Pythium* root rot of sugar cane, and that of Heck (17) on the relation of low phosphorus and high mineral nitrogen to the same disease, have a direct bearing on the browning problem, but will not be discussed at this point as they were published after these investigations were completed. The fact that Heck's interesting results and those submitted in this paper were arrived at independently should add weight to the conclusions presented in both papers. The similarity of the nutrient factors affecting *Pythium* root rot of wheat and of those affecting *Pythium* root rot of sugar cane as reported by Cooke (11), Heck (17), and more recently by Carpenter (8), are pointed out in the general discussion.

In view of the above considerations regarding unbalanced nitrogen and phosphorus metabolism, it seemed advisable to analyze soil samples from browning-infested patches and apparently healthy patches in the same field for nitrate nitrogen and available phosphorus. In conjunction with this a study of the effects of nitrogenous and phosphatic fertilizers mainly, on wheat seedlings grown in crocks in browning or "diseased" and in normal or "healthy" soils, was also conducted. In this paper "diseased" soil and "healthy" soil shall be taken to mean soil which produces diseased and relatively healthy seedlings respectively.

Available Phosphorus and Nitrate Nitrogen of *Pythium*-infested Fields

The collection of soil samples from areas with diseased plants and from areas of healthy plants began with the first appearance of the disease in June. Samples were obtained only from fields showing characteristic pronounced symptoms of the disease. These were situated over a wide range of territory in central and north-central Saskatchewan. A thorough washing and examination of the roots in a large white porcelain dish facilitated and insured a correct diagnosis in the field. Each soil sample for analysis was a composite of eight samples taken to a depth of six inches from between the rows and thoroughly mixed on an oilcloth. Thus, from each study field one composite sample of soil from diseased and one from apparently healthy areas were obtained. The samples were placed in quart sealer jars and 4 cc. of toluene added to each, in order to stop nitrification and other bacterial action. The samples were thoroughly air dried in the laboratory within 48 hours of collecting, and then stored in the jars until the chemical analyses were performed about a fortnight later. It was considered that no appreciable change in the nitrate nitrogen content would occur in the air-dried soil. A slight modification of the Truog method, as employed by Mitchell (29), was used for the estimation of available phosphorus, and Harper's modification (16) of the Colorimetric Phenoldisulphonic Acid method for the estimation of nitrate nitrogen.*

Table I shows the results of analyses of soil samples from diseased and healthy areas of wheat on summerfallowed land for June, 1932, and Table II the results for June, 1933.

TABLE I

ANALYSES OF SOIL SAMPLES FROM DISEASED AND HEALTHY AREAS OF WHEAT ON SUMMERFALLOWED LAND, JUNE 1932

Field	Locality (town)	Date collected	Available P, p.p.m.		NO_3, p.p.m.		pH, Electrometric		Soil texture
			Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	
1	Saskatoon	June 14	36	78	17.2	10.9	7.30	7.92	Loam, silty
2	Environ	14	55.5	82	12.9	1.7	7.07	8.02	Loam
3	Wilkie	17	23.5	52	17.8	7.5	7.36	8.17	Loam
4	Scott	17	15	42	16.6	1.6	7.33	7.85	Loam
5	Saskatoon	18	19	56	4.4	11.6	6.67	8.07	Loam
6	Bruno	20	53	103	1.2	1.1	7.42	7.93	Heavy loam
7	Kinistino	21	82	139	4.6	3.9	6.95	7.26	Heavy loam
8	Aberdeen	21	15	63	14.1	6.8	7.65	8.01	Loam
9	Prudhomme	21	38	49	10.8	1.2	7.71	7.42	Loam, silty
10	Dana	21	31.5	77	24.2	23.4	7.20	7.62	Loam, silty
11	Bremen	21	34.5	60	25.7	7.8	—	—	Loam, silty
12	Wakaw	21	20.5	64	4.1	1.4	—	—	Loam, silty
13	Domremy	21	42	72	5.1	2.5	—	—	Loam, smooth
14	Duck Lake	22	23	54	4.3	0.7	—	—	Loam, smooth
15	Waldheim	22	25	49	13.2	2.0	—	—	Loam, light
16	Sutherland	27	90	84	2.3	4.4	—	—	Clay loam
17	Saskatoon	27	32	80	3.4	2.2	—	—	Clay loam
18	Saskatoon	27	30.5	45.5	2.4	2.6	—	—	Loam
19	Lanigan	28	53	51.5	0.3	0.2	—	—	Loam, heavy
20	Lanigan	28	35	52	0.1	0.2	—	—	Fine sandy loam
21	Lanigan	28	24	28	0.3	0.3	—	—	Fine sandy loam
22	Humboldt	28	24	49	4.6	0.6	—	—	Loam
Mean			36.5	65.0	8.6	4.3	7.26	7.82	

The results in Tables I and II are, in the main, similar and may be considered together. In both it will be observed that, with few exceptions, available phosphorus is less in diseased areas than in healthy, while nitrate nitrogen is greater in diseased areas than in healthy. This relation may not have prevailed at the time of seeding, but relevant work (9, 10, 38, 39, 43) supports the view that plants absorb nutrients during the early stages of growth in approximately the same proportion in which these elements are present in

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**These determinations were made by Frank Riecken, M.Sc., formerly of the Soils Department, to whom my thanks are due.*

TABLE II

ANALYSES OF SOIL SAMPLES FROM DISEASED AND HEALTHY AREAS OF WHEAT ON SUMMERFALLOWED LAND, JUNE, 1933

Field	Locality (town)	Date collected	P, p.p.m.		NO_3, p.p.m.		pH		Soil texture
			Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	
1	Saskatoon	June 6	29	40	6.0	5.9	7.70	7.60	Loam
2	Saskatoon		30	59	10.9	4.3	6.80	6.75	Loam
3	Saskatoon	June 8	56	160	24.0	24.4	8.10	7.64	Clay
4	Saskatoon		36	39	8.7	7.3	7.42	6.80	Loam
5	Saskatoon		28	46	12.0	16.0	7.15	7.44	Loam
6	Saskatoon		23	17	17.2	9.6	6.00	7.60	Loam
7	Saskatoon	June 9	64	33	30.0	27.7	6.44	7.62	Loam (heavy)
8	Saskatoon		54	78	11.4	10.1	6.30	7.80	Loam
9	Vanscoy		38	47	36.2	38.0	6.65	7.07	Loam
10	Saskatoon		28	57	25.0	19.7	6.30	7.70	Loam (silty)
11	Clavet	June 13	33	63	20.2	9.2	6.35	7.70	Clay loam
12	Elstow		47	96	19.7	18.5	6.36	7.16	Silty clay
13	Lanigan		34	37	3.0	1.3	7.90	7.90	Loam
14	Sheho		37	56	4.5	3.4	8.00	7.74	Loam
15	Yorkton		31	81	17.5	5.6	8.08	8.10	Hy. silt loam
16	Yorkton		41	43	30.4	19.9	8.00	7.10	Hy. silt loam
17	Yorkton		24	62	7.1	1.8	8.00	8.16	Loam
18	Yorkton		18	36	9.3	2.6	8.30	8.10	Loam
19	Quill Lake	June 14	27	41	4.3	4.5	7.45	7.76	Loam
20	?		32	64	10.6	6.0	7.76	7.77	Loam
21	Englefeld		24	46	3.1	3.0	8.08	7.50	Light loam
22	St. Gregor		33	35	15.9	13.4	7.92	7.63	Loam
23	Munster		31	41	27.3	33.9	7.77	7.75	Loam
24	Humboldt		27	39	19.8	5.7	6.91	6.85	Loam
25	Humboldt		31	68	29.8	12.9	6.33	7.52	Loam
26	Aberdeen	June 15	28	56	5.9	5.9	8.17	8.10	Clay loam
27	Saskatoon	June 17	33	68	15.4	10.2	6.92	7.45	Loam
28	Saskatoon		23	42	22.4	32.7	5.90	6.40	Loam
29	Sonningdale		26	38	24.8	18.9	6.15	6.59	Loam
30	Sonningdale		26	62	29.5	13.8	6.14	7.85	Loam
31	Sonningdale		23	23	15.3	3.5	6.34	6.59	Loam
32	Sonningdale		25	53	25.8	22.5	6.51	6.75	Loam
33	Scott	June 20	15	14	21.6	5.0	5.50	5.75	Heavy loam
34	Unity		14	44	15.2	9.8	5.55	7.10	Loam
35	Unity		16	18	28.8	9.2	5.40	5.45	Loam
36	Unity		17	40	22.5	62.0	5.49	6.25	Loam
37	Wilkie		18	51	33.0	43.5	5.65	7.35	Loam
38	Brandon		65	48	44.0	13.4	7.85	8.10	Clay loam
39	Prudhomme	June 22	34	41	22.2	16.4	7.93	7.85	Loam
40	Dana		20	50	92.5	15.9	6.50	7.61	Loam
41	Wakaw		26	49	6.2	22.2	6.95	7.85	Loam (silty)
42	Domremy		35	68	12.9	2.6	6.93	7.60	Loam (silty)
43	St. Isidore		34	48	3.8	7.5	7.35	7.50	Loam
44	Rosthern		18	64	13.1	13.2	6.20	7.62	Heavy loam
Mean			30.7	51.4	19.5	14.4	6.95	7.37	
Mean, 1932-1933			33.6	58.2	14.1	9.4	7.10	7.59	

the soil. It is, however, reasonable to expect a greater number of irregular results with nitrates than with available phosphorus, as shown in Tables I and II, because of the greater tendency of nitrates to fluctuate with changing biological and physical conditions of the soil and with the movement of the soil moisture. The pH of the soil solution from diseased areas is usually slightly lower than that from healthy areas, but no importance is attached to this difference. All of the results appear to be independent of soil type. The available phosphorus and nitrate nitrogen results are presented graphically in Figs. 1 and 2.

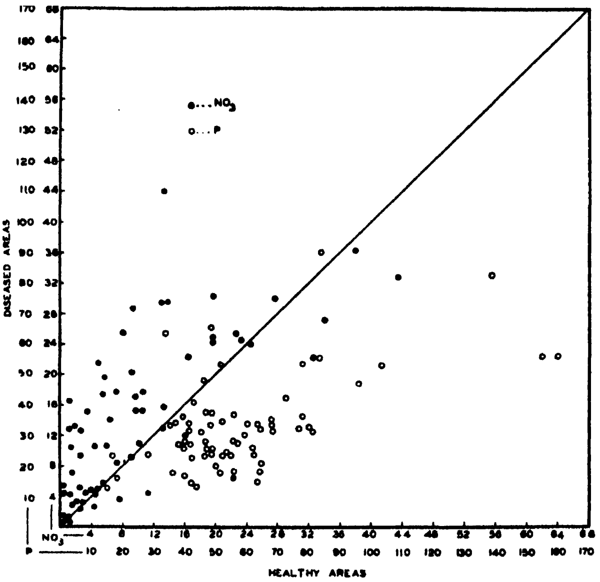


FIG. I

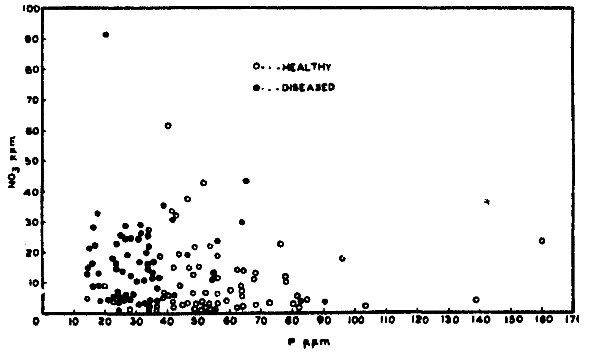


FIG. 2

FIGS. 1 and 2. *Showing graphically the distribution of the fields according to the results in Tables I and II.*

The presence of soluble salts in the soil solution is considered by McGeorge and Breazeale (27) to depress phosphate solubility, and Sideris and co-workers (40) have mentioned that in high salt concentrations pineapples use the oldest leaves as storage organs for the excess of salt. However, tests showed soluble salts to be present in very small amounts in the soil samples of Table II.

Fields in Relation to Available Phosphorus and Nitrate Nitrogen Ratios

In Table III, the 66 fields are arranged according to their relative content of available phosphorus and nitrate nitrogen as shown in Tables I and II. In 65% of the fields the diseased areas contain less available phosphorus and more nitrate nitrogen than healthy areas; 21% show low available phosphorus associated with low nitrate nitrogen; 7.5% show high available phosphorus associated with high nitrate nitrogen, and only 1.5% show high available phosphorus associated with low nitrate nitrogen. Ninety per cent of diseased fields are lower in available phosphorus than healthy fields, and 74% of diseased fields are higher in nitrate nitrogen than healthy fields. Thus viewed, the results indicate more clearly the probable correlation between less available phosphorus and more nitrate nitrogen in browning than in normal areas, and suggest that low available phosphorus is possibly more closely correlated with the disease than high nitrate, if the two can be considered separately. As already pointed out, however, the nitrate differences might have been greater during the first four weeks of growth before disease symptoms appeared.

TABLE III

Number of fields according to available phosphorus and nitrate nitrogen ratios of the soil in diseased and healthy wheat patches for 1932 and 1933

		No. of fields in which available phosphorus in			
	Ratio	D* < H	D > H	D = H	
No. of fields in which nitrate nitrogen in	D > H	43	5	1	49
	D < H	14	1	—	15
	D = H	2	—	—	2
		59	6	1	66

*D *represents diseased wheat areas, and* H, *healthy wheat areas.*

Nutrients in Browning Fields as a Percentage of Nutrients in Healthy Fields

When the nutrients in each browning field are expressed as percentages of the nutrients in each normal field and the mean taken, it is found that for 1932, the amount of available phosphorus is 56.5% and of nitrate nitrogen 324.3%; and for 1933, 67.1% and 188.1% respectively. These are shown diagrammatically in Fig. 3. They suggest a possible reciprocal relation between available phosphorus and nitrate nitrogen; an increase in available phosphorus being accompanied by a decrease in nitrate nitrogen and *vice versa*.

Such a relation has been recognized by many investigators (19, 20, 24, 25). It should be borne in mind, however, that nitrates might fluctuate a great deal and frequently accumulate to a great extent in soils that are high in available phosphorus.

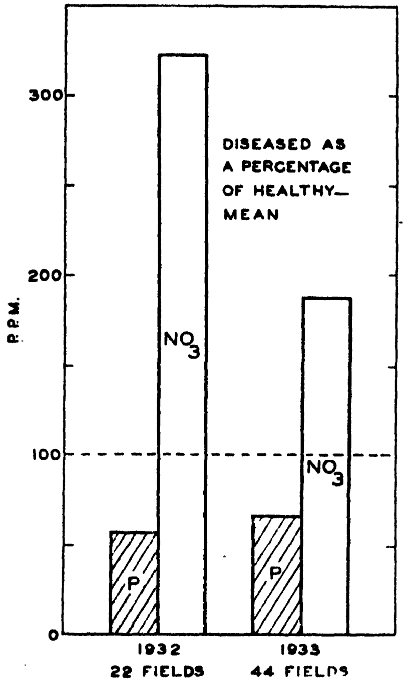


FIG. 3. *Available phosphorus and nitrate nitrogen in browning fields expressed as percentages of available phosphorus and nitrate nitrogen in normal fields, 1932 and 1933.*

Soil Nutrients and Plant Growth

In Table IV are shown the relative heights of seedlings in June, in diseased and healthy areas, together with the corresponding available phosphorus and nitrate nitrogen of the soil. The average height of the diseased seedlings is 7.8 cm. less than that of healthy seedlings (those sections receiving phosphate applications in Fields 34 and 35 are not included), the diseased condition being associated, as before, with less available phosphorus and more nitrate nitrogen than the healthy condition. In Fields 5 and 33, when the diseased areas of the fallow crop are compared with the healthy areas of the stubble

TABLE IV

HEIGHT OF WHEAT SEEDLINGS AT APPROXIMATELY SIX WEEKS IN RELATION TO AVAILABLE PHOSPHORUS AND NITRATE NITROGEN CONTENT OF SOIL AND TO CULTIVATION PRACTICE, JUNE 1933

Field	Location (town)	Treatment	Condition	Height, cm.	P, p.p.m.	NO_3, p.p.m.
2	Saskatoon	Fallowed	Healthy	33	59	4.3
		Fallowed	Diseased	23	30	10.9
5	Saskatoon	Fallowed	Healthy	34	46	16.0
		Fallowed	Diseased	24	28	12.0
		Stubble crop	Healthy	31	30	3.5
31	Sonningdale	Fallowed	Healthy	24	23	3.5
		Fallowed	Diseased	20	23	15.3
33	Scott	Fallowed	Diseased	22	15	21.6
		Spring ploughed	Healthy	22	14	5.0
34	Unity	Fallowed	Healthy	30	44	9.8
		Fallowed	Diseased	26	14	15.2
		Ploughed in autumn before fallow	Healthy	33	31	4.1
		Fallow + P	Healthy	42	—	—
35	Unity	Fallowed	Diseased	22	16	28.8
		Fallowed + P	Healthy	38	18	9.2
41	Wakaw	Fallowed	Healthy	37	49	22.2
		Fallowed	Diseased	29	26	6.0
42	Domremy	Fallowed	Healthy	42	68	2.6
		Fallowed	Diseased	30	35	12.9
43	St. Isidore	Fallowed	Healthy	37	48	7.5
		Fallowed	Diseased	27	34	3.0
44	Rosthern	Fallowed	Healthy	31	64	13.2
		Fallowed	Diseased	22	18	13.1

crop, the available phosphorus content of the soil in the respective areas is seen to be practically the same, while the nitrate nitrogen is high in both cases in the diseased fallow-crop area, *i.e.*, the closer P/N ratio in both cases is associated with the diseased condition.

Special attention should be drawn to Fields 31 and 34. In Field 31, the diseased area was bare fallowed during the whole summer of 1932, while the healthy area carried a crop of wheat and oats in 1932 which was cut for green feed in mid July. The latter area was not ploughed until mid August. The difference in the condition of the seedlings on the two portions of the same field could clearly be observed from some distance. In Field 34, all portions received the same fallow treatment in 1932, but one wide strip was ploughed shallow in the autumn of 1931 to turn under a heavy growth of weeds. This strip carried a uniformly healthy crop in 1933, while the remainder of the field, with the exception of the strip receiving phosphate fertilizer, was spotted with diseased and healthy areas (Fig. 4). It is possible that decomposing organic matter of the "weedy" strip with its concomitant evolution of carbon dioxide increased the availability of phosphorus as suggested previously (15, 28, 42), while the nitrogen removed by the weeds produced a relative decrease in the available nitrate in the soil (2).

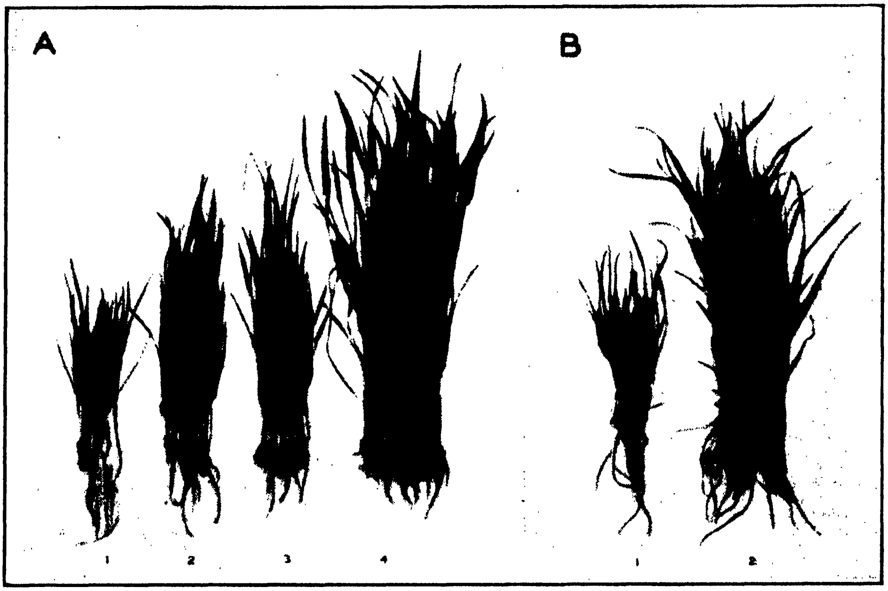


FIG. 4. *Wheat following summerfallow; 20 plants to each bundle.* A, *field 34, Table IV, 1, plants showing browning symptoms; 2, normally healthy plants; 3, heatthy plants from strip on which weeds were ploughed under the autumn preceding summerfallow, and 4, triple superphosphate treated plants.* B, *field 35, Table IV, 1, untreated diseased plants, and 2, plants receiving triple superphosphate.*

Relation between Soil Nutrients, Height at Maturity, and Date of Maturity

Eleven of the fields selected for study in June, 1932, *viz.*, numbers 1, 2, 5, 6, 8 to 10, 15 to 18 (Table I), were visited in August just before harvest and, with the exception of Field 16, the diseased wheat was a few to 20 cm. shorter and from 3 to 21 days later in maturing than the healthy wheat. Similar conditions obtained in fields under observation in 1933 and 1934. This delayed maturity might be attributed, in part at least, to the higher nitrate content of the soil. Field 15 (Table V) showed a slight difference in height but there was no appreciable difference in date of maturity. In the case of Field 16 (Table I), the exception referred to above, there was no difference in height of plants or in date of maturity between what were diagnosed in June as diseased and as healthy patches, respectively. Data on four of these fields are presented in Table V.

TABLE V

RELATION BETWEEN AVAILABLE PHOSPHORUS AND NITRATE NITROGEN CONTENT OF SOIL, HEIGHT OF PLANTS AT MATURITY, AND DATE OF MATURITY, 1932

Field	Locality (town)	Crop condition in June	Soil analyses, June		Height on Aug. 12, cm.	Estimated delay in maturity of diseased plants
			P, p.p.m.	NO_3, p.p.m.		
8	Aberdeen	Healthy Diseased	63 15	6.8 14.1	82 77	3 to 4 days
9	Prudhomme	Healthy Diseased	49 38	1.2 10.8	101 84	10 to 14 days
10	Dana	Healthy Diseased	77 31.5	23.4 24.2	99 79	14 days
15	Waldheim	Healthy Diseased	49 25	2.0 13.2	99 96	No appreciable difference

In general, it appears that a narrow P/N ratio in the available soil solution early in the season allows the disease to become manifest and delays maturity, whereas a wide P/N ratio maintains the crop in apparently healthy condition and hastens maturity. At the same time it might be possible to have a narrow ratio in a normal soil which was high both in available phosphorus and nitrate without the seedlings becoming susceptible to fungal attack. Since both diseased and healthy areas in each field have received the same cultural treatment, it may be inferred that the differences in available nutrients are due to inherent soil factors or to the activities of micro-organisms.

Expected Response to Phosphatic Fertilizers

The question arises, can an increase in yield from phosphate applications be expected on the browning areas which are relatively low in available phosphorus? Mitchell (29) concluded from his findings for Saskatchewan soils generally "that the soils lighter than clays where the "availability" of phosphorus is below 20 parts per million as found by this method (modification of Truog's) large increases in grain yield may be expected from phosphate treatments of the soil. With an availability of from 20 to 40 parts per million a response is still probable, while at 50 to 60 parts per million "available" phosphorus, the point is reached where little or no response is likely". On the basis of his findings, the data in Tables I and II have been arranged to show the distribution of fields in relation to expected yields from phosphate applications based on available phosphorus in the soils. Three arbitrary divisions have been recognized in accordance with Mitchell's data. They are considered significant only in so far as they allow us to say that a soil is well supplied or moderately supplied with available phosphorus or is deficient in it. All fields with more than 45 p.p.m. available phosphorus are considered *unlikely* to give increases in yields; from those with 21 to 45 p.p.m. available phosphorus, increases in yields are *probable*, and from those with less then 20 p.p.m. available phosphorus, *large* increases in yields may be expected.

Table VI gives the distribution of fields in relation to expected yields from phosphate applications based on available phosphorus in soils, and Table VII presents the percentage distribution.

TABLE VI

DISTRIBUTION OF FIELDS IN RELATION TO EXPECTED YIELDS FROM PHOSPHATE APPLICATIONS BASED ON AVAILABLE PHOSPHORUS IN SOILS

Year	Healthy areas			Diseased areas			Total no. of fields
	Increase in yields			Increase in yields			
	Large. (P, p.p.m. 20 or less)	Probable. (P, p.p.m. 21 to 45)	Unlikely. (P, p.p.m. 46 or more)	Large. (P, p.p.m. 20 or less)	Probable. (P, p.p.m. 21 to 45)	Unlikely. (P, p.p.m. 46 or more)	
1932	0	2	20	3	14	5	22
1933	3	16	25	8	31	5	44
1932-1933	3	18	45	11	45	10	66

TABLE VII

PERCENTAGE DISTRIBUTION OF FIELDS IN RELATION TO EXPECTED YIELDS FROM PHOSPHATE APPLICATIONS BASED ON AVAILABLE PHOSPHORUS IN SOILS

Year	Response to P likely. P, 45 p.p.m. or less		Response to P problematical. P, 46 p.p.m. or more	
	Healthy	Diseased	Healthy	Diseased
1932	9.1	77.3	90.9	22.7
1933	43.2	88.6	56.8	11.3
1932–1933	31.8	84.8	68.1	15.1

It will be seen from the 66 fields studied that the greatest response to phosphate applications is likely to occur in the diseased areas, *viz.*, 84.8%, while only 31.8% of the healthy areas are likely to give increases in yield. These results aid in explaining: first, the general uniformity in wheat fields in browning root rot districts which have received phosphate fertilizer, compared with the unevenness in height and stage of maturity in those untreated; and second, the results of the fertilizer experiments conducted on browning and normal soil in the greenhouse and reported in Section IV.

It is recognized that the method of ascertaining the "available" phosphorus in a given soil and using this as a guide in predicting the probable response to phosphate of a wheat crop grown thereon has decided limitations and is not infallible, but the results in this province and elsewhere have more often than not shown a close correlation. In recent years there have been rapid developments of both chemical and biological methods for diagnosing nutrient deficiencies in soils and of basing recommendations for the use of fertilizers on the results obtained. The Truog method of ascertaining the "available" phosphorus in a given soil has provided, in a large majority of cases, a fair basis for predicting the probable response to phosphate fertilizers.

Browning Root Rot in Relation to Cultivation Practices and Available Phosphorus and Nitrate Content of the Soil

The fields chosen for this study were partly in fallow crop and partly in a fall- or spring-ploughed stubble crop. The burned straw-stack area and the gray soil area were in zones of fallow crop. The results of the determinations are given in Table VIII.

TABLE VIII

ANALYSES OF SOIL SAMPLES IN RELATION TO VARIOUS CULTIVATION PRACTICES AND *Pythium* ROOT ROT OF WHEAT

Locality (town)	Date collected	Soil preparation	Condition of crop	"Available" P, p.p.m.	NO_3, p.p.m.
1932:					
Sonningdale	June 14	Following fallow	Diseased	38	10.6
		Following wheat—fall ploughing	Healthy	35	0.9
		Burned straw-stack zone—fallow, 1931	Healthy	129	2.3
Scott	June 17	Following fallow	Diseased	15	16.6
		Following wheat—spring ploughing	Healthy	19	0.6
		Spot of gray soil—fallow, 1931	Healthy	42	1.6
1933:					
Saskatoon	June 8	Following fallow	Diseased	28	12.0
		Following wheat	Healthy	30	3.5
Scott	June 20	Following fallow	Diseased	15	21.6
		Following wheat—spring ploughing	Healthy	14	5.0

The results show that in the same field the amount of available phosphorus is practically the same in the "diseased" fallow-crop areas as in the normal fall-or spring-ploughed areas (*cf.* 29), while the nitrate nitrogen is considerably higher in the "diseased" fallow-crop areas. That is, where the P/N ratio is relatively wide the crop is apparently healthy, and conversely where it is narrow the crop is diseased. Thus it appears that cultivation practices can have a determining influence on the incidence of the disease.

In the Sonningdale field where the burned straw and chaff ashes have returned much available phosphorus to the soil, a healthy crop develops. The wide P/N ratio prevailing in the gray spot in the Scott field also permits a healthy condition.

It is possible that the nitrate in the diseased areas was not initially higher than in the healthy, but became so because of the incapability of the damaged root system to absorb and utilize efficiently the nitrate already present. However, this does not seem likely in these instances as fallowed soil is invariably higher in nitrate than stubble soil. Similarly, it could be argued that the healthy plants would also tend to absorb more phosphorus and reduce the available supply relative to the diseased area, but there is still ordinarily about twice as much available phosphorus in the healthy area in spite of the greater absorption (Table I).

Burned Straw-stack Areas

The better growth and yield on areas which formerly carried straw stacks which were subsequently burned is carried over for many years in most cases. Soil samples from three such zones were included in the analyses, the results of which are presented in Table IX.

TABLE IX

Analyses of soil samples from old burned straw-pile zones. Wheat following fallow

Locality (town)	Condition of crop	"Available" P, p.p.m.	NO_3, p.p.m.	pH
1932:				
Sonningdale	Healthy	129	2.3	8.11
1933:				
Saskatoon	Healthy	220	9.2	8.60
Saskatoon	Healthy	126	12.4	7.50
Mean		158	7.9	8.07

The results indicate that the good growth is to be accounted for by the extremely high available phosphorus content of the soil, coupled with a sufficiency of the other essential elements, and not by a high nitrate content as is commonly assumed on the prairies. The nitrate is usually of an order comparable to that of many normally healthy areas in the same field. The kind of cereal doubtless has an influence on the amount of phosphorus present; for example, wheat straw and chaff will supply more phosphorus than oat straw and chaff (18). As reported later under the section on fertilizer treatments, wheat in browning soil, which is naturally lower in available phosphorus and higher in nitrate nitrogen than normal soil, responds well to cereal-straw ashes, while in the normal soil there is little or no response.

Miscellaneous Considerations

1. *Dark- and light-colored soil areas.* In some browning root-rot districts, especially in north-central Saskatchewan, there is considerable variation in soil color in the same field due mainly to the removal of some surface soil by wind erosion or to the existence at one time of lightly wooded areas. To ascertain whether there was any correlation between these areas and browning root rot, 35 fallow fields, or parts thereof, were charted for dark- and light-colored areas in the autumn of 1932. An examination of the young wheat crop the following June showed no definite correlation between color and the presence of the disease except in two instances. In general, therefore, there seems to be no relation between soil color and the disease in a given field.

2. *Iron and manganese.* It is well known that many alkaline soils have a tendency to fix iron and manganese so that these elements become almost unavailable to plants. In preliminary pot experiments on the application of iron and manganese compounds to browning root-rot soil no difference in the growth of wheat could be recognized between the treated plants and the checks.

IV. EFFECTS OF FERTILIZER AMENDMENTS

The ratios of available phosphorus and nitrate nitrogen in browning and normal soil, as revealed by the chemical analyses, made necessary a study of the effects of fertilizers, especially phosphatic and nitrogenous, on browning root rot of wheat. Consequently, throughout this section on fertilizers it should be borne in mind that browning or "diseased" soil is relatively lower in available phosphorus and higher in nitrate nitrogen than normal or "healthy" soil.

Fertilizer experiments were conducted in the greenhouse in soil which had been collected during the summer from diseased and healthy areas respectively, in infested fields. The samples were air dried as soon as possible after collecting. This was followed by a thorough mixing of the "diseased" samples to form one composite and of the "healthy" samples to form another, the two lots being then stored in this condition until used for experimentation two or three months later.

Preliminary experiments on wheat seedlings up to about six weeks, the stage at which browning symptoms are most striking in the field, showed that potassic fertilizers produced no differences on growth when applied either to diseased or to healthy soil; consequently no further tests with potassic fertilizers were considered necessary. Subsequent experiments were on the effects of phosphatic and nitrogenous fertilizers mainly.

Fertilizer Applications

All phosphatic fertilizers were applied at seed level at the rate of 1 gm. in Expts. 1 to 3, and 0.5 gm. in subsequent experiments, to each six-inch crock or pot at the time of sowing. Sodium nitrate was applied in solution at the rate of 1.5 gm. to each six-inch crock in the earlier and 0.75 gm. in the later experiments. Calcium nitrate was applied in amounts containing equivalent quantities of nitrate to the sodium nitrate application. The solution aliquots were applied at intervals, the first at the time of sowing, the second after two weeks, and the remaining portion after four weeks. The relative position of each crock on the greenhouse bench was changed at frequent intervals. Marquis spring wheat was used throughout the investigation.

Experiment 1. This experiment was conducted in duplicate. The plants were watered every second or third day with approximately the same amount of distilled water. Sowings were made on October 30, 1932, and the plants harvested 54 days afterwards (Fig. 5).

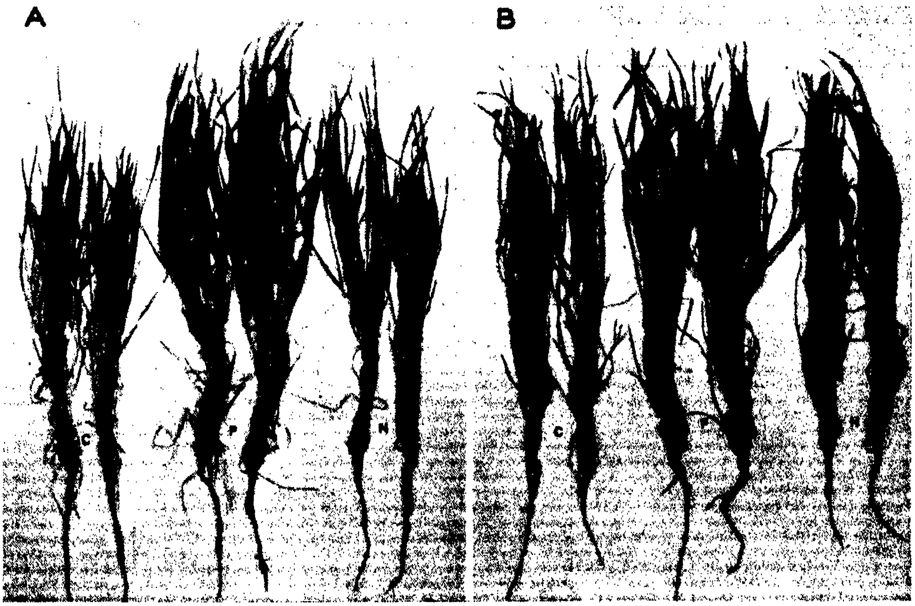


FIG. 5. *Showing response of wheat on* A, *browning soil, and* B, *normal soil, to phosphatic and nitrogenous fertilizers in a duplicate pot experiment; ten plants to each bundle.* C, *untreated control;* P, *receiving triple superphosphate, and* N, *receiving sodium nitrate. See Expt. 1, text and Table X.*

The results of Expt. 1 (Table X) show that in the untreated healthy soil there is approximately 50% increase in the oven-dried weight of the seedlings over those in the untreated diseased soil. In diseased soil the triple superphosphate dressing gave an increase of approximately 125%, while the nitrate fertilizers had no appreciable effect. In the healthy soil the phosphate dressing gave an increase of approximately 33%, and the nitrate fertilizers again had no appreciable effect. This increased response to phosphate of diseased soil compared with healthy was not unexpected, since the diseased soil to begin with contained only about half as much available phosphorus as the

TABLE X

RESPONSE OF WHEAT ON SOIL FROM DISEASED AND HEALTHY AREAS TO VARIOUS FERTILIZER TREATMENTS

Experiment 1—54 days

Treatment	"Diseased" soil (mixture)			"Healthy" soil (mixture)		
	No. of plants	Average height, cm.	Oven-dry weight, gm.	No. of plants	Average height, cm.	Oven-dry weight, gm.
None—control	20	40	2.086	20	45	3.108
Triple superphosphate	19	50.7	4.701	20	47.7	4.283
Sodium nitrate	20	41.5	2.103	20	44.5	2.993
Calcium nitrate	20	42	2.238	19	44.5	2.881

Experiment 2—54 days

	"Diseased" soil* (single field)			"Healthy" burned straw-bottom soil†		
None—control	10	45	1.304	10	54	2.415
Triple superphosphate	10	46	1.685	10	50	2.325
Sodium nitrate	10	40	1.024	10	50	2.755
Calcium nitrate	10	41.5	1.098	10	53	2.509
Ashes	10	43	1.642			

Experiment 3—45 days

	"Diseased" soil (mixture)					
None—control	20	40	2.431			
Triple superphosphate	18	47	5.332			
Ammonium phosphate	20	44.5	6.393			
Sodium nitrate	20	41.5	2.709			
Straw	20	43.5	3.349			
Ashes	20	46.0	4.794			

Experiment 4—60 days

	"Diseased" soil (mixture)			"Healthy" soil (mixture)		
None—control	30	45	3.209	30	45	4.852
Triple superphosphate	30	46	7.139	30	48	7.248
Sodium nitrate	30	47	3.941	29	45	4.411

**Originally contained 10.6 p.p.m. nitrate nitrogen and 38 p.p.m. available phosphorus.*

†Originally contained 2.3 p.p.m. nitrate nitrogen and 129 p.p.m. available phosphorus.

healthy soil. It, however, contained about twice as much nitrate. The lack of response of the healthy soil to nitrate applications indicates that nitrate was not in this case a limiting factor though it was originally present in less amounts than in diseased soil. The possibility for favorable conditions for nitrification in this pot-culture work under greenhouse conditions must not be overlooked.

Experiment 2. This was conducted in one series on soil obtained from a single badly infested field, but otherwise in a manner similar to Expt. 1. The ashes were obtained from burned wheat straw and chaff and were applied at the rate of 5 gm. per crock. The determinations made on the samples collected in June showed that the diseased areas contained 38 p.p.m. available phosphorus and 10.6 p.p.m. nitrate nitrogen, while the healthy areas, where old straw stacks had been burned some two years previously, contained 129 p.p.m. available phosphorus and 2.3 p.p.m. nitrate nitrogen. The wheat plants on the healthy areas averaged 10 to 15 cm. taller than adjoining diseased wheat at harvest, and were 10 to 14 days earlier in maturing.

The results of Expt. 2 (Table X) showed that on the diseased soil, nitrate dressings caused a moderate decrease, while both phosphate and ashes caused a moderate increase over the untreated controls; on the healthy soil, nitrate produced a slight increase and phosphate a slight decrease. The original available phosphorus and nitrate contents of the soil samples probably offer a satisfactory explanation.

Experiment 3. Tap water was used for watering the plants in this and subsequent experiments as it was found that it contained negligible quantities of phosphate and nitrate. The straw amendment consisted of a small unmeasured amount of finely chopped straw which was thoroughly incorporated with the top three inches of soil in the two crocks a few days previous to sowing. Plate I, Fig. 1, shows the plants when harvested. Each bundle consists of two pot replicates of ten plants each.

From the data of Expt. 3 (Table X), it will be noticed that the phosphate applications again gave well over 100% increase in dry weight over the untreated control. A slight increase from nitrate is noticed. The straw, however, has given a substantial increase, while the ashes almost doubled the yield. The beneficial effect of the ashes as shown in Expts. 2 and 3 is probably due more to the phosphate than to the potash content, since, as previously mentioned, wheat on the open prairie soils was not benefited by potassic fertilizers.

Experiment 4. This experiment was conducted in triplicate in composite soil samples collected from 44 fields in 1933. Sowings were made on October 16, and the plants harvested 60 days afterwards.

These results are, in the main, in general agreement with those of Expt. 1 which was conducted on composite samples collected the previous year.

The data from Table X must also be viewed from the standpoint of increased or decreased resistance or liability of the host plant to fungal invasion. Plants receiving phosphate amendments or ashes showed no aboveground symptoms of browning, but the nitrate-treated pots had as many discolored leaves as the controls. Pots with straw varied in this regard, depending presumably upon the degree of decomposition of the straw and its effect on the nitrate content of the soil. Typical *Pythium* root-tip lesions were found on the phosphate-treated plants, but the root systems as a whole had a decidedly white appearance while those of the nitrate-treated plants and the controls were quite yellow in contrast owing to the invasion of more fine laterals and cortical tissue by the fungus.

Experiments 5 and 6. Attempts were made in these experiments (Table XI) to determine the effects of various fertilizer dressings on the development and number of diseased crown roots, in diseased and healthy soil respectively.

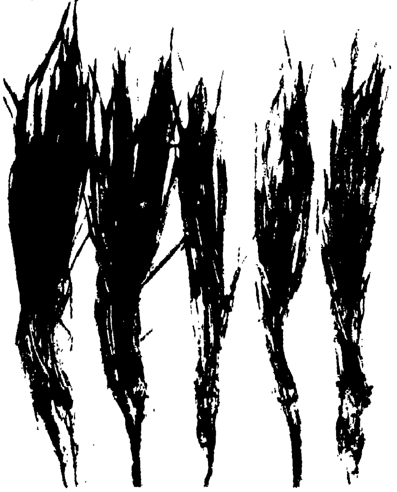
TABLE XI

EFFECT OF SOIL AMENDMENTS ON THE DISEASE, WITH SPECIAL EMPHASIS ON CROWN-ROOT DEVELOPMENT

Treatment	No. of plants	Height, cm.	Oven-dry weight, gm.	Crown roots				
				Total no.	Length, cm.	% Healthy	% Diseased	H/D ratio
Experiment 5—41 days								
"Diseased" soil:								
None—control	30	37	4.854	66	—	44.0	56.0	0.8
Triple superphosphate	30	42	9.104	135	—	64.5	35.5	1.8
Sodium nitrate	30	36	4.986	66	—	27.2	72.8	0.37
"Healthy" soil:								
None—control	30	39	6.339	102	—	46.1	53.9	0.85
Triple superphosphate	30	39.5	8.356	131	—	66.4	33.6	1.9
Sodium nitrate	30	39	7.704	109	—	58.7	41.3	1.4
Experiment 6								
Soil I—"diseased":								
None—control	30	34	3.127	90	269	56.6	43.4	1.31
Triple superphosphate	30	37	5.595	135	695	60.7	39.3	1.56
Ammonium phosphate	30	38	5.572	133	568	63.1	36.9	1.71
Ammonium sulphate	30	36	3.632	83	143	57.8	42.2	1.37
Manure—12 tons per acre	30	38	5.554	151	515	45.7	54.3	0.84
Soil II—"diseased":								
None—control	28	—	2.880	93	—	9.7	90.3	0.11
Manure—12 tons per acre	28	—	4.540	177	—	31.6	68.4	0.46

The seeds were sown 2.5 in. deep to permit the development of a long sub-crown internode, which facilitated the separation of seminal and crown roots after careful and thorough washing in running water at the time of harvesting. No satisfactory methods of obtaining a disease rating of plants affected with *Pythium* root rot had been worked out, principally because of the difficulty of estimating the diseased condition of the very fine lateral roots which may show no sign of a diseased condition to the unaided eye, but which the microscope reveals to be filled with *Pythium* mycelium and generally some oospores as well. This destruction of the fine laterals is important since browning root-rot symptoms appear in some fields before the crown roots have developed. In such instances no severe lesioning may exist on the main primary roots, though the whole of the primary root system is yellower in mass than that of a healthy plant.

1



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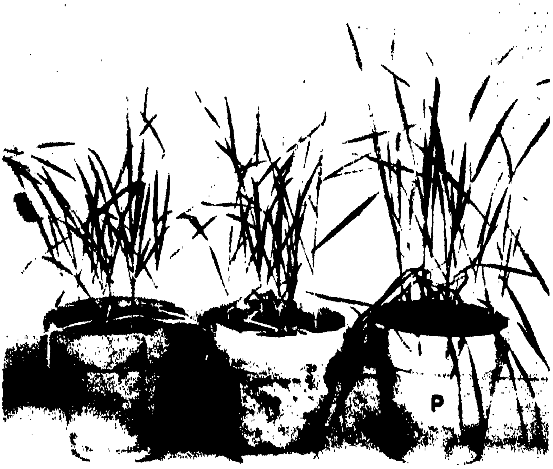

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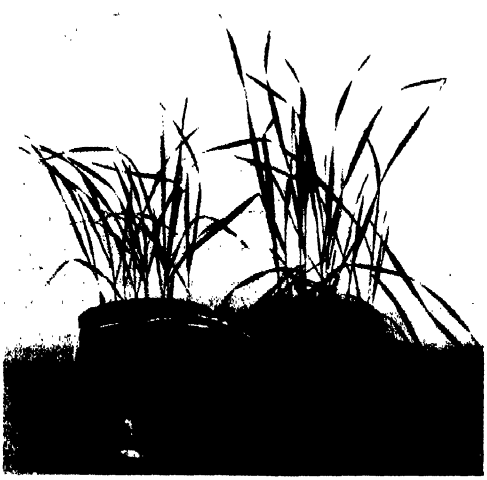

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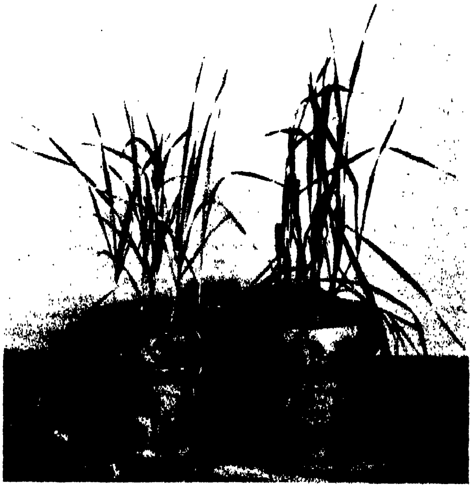

FIG. 1. *Six weeks old wheat seedlings in browning soil receiving the following treatments, left to right: triple superphosphate, ammonium phosphate, untreated control, sodium nitrate, and straw. See Expt. 3, text and Table X.* FIGS. 2 to 4. *Plants from Expt. 5, see text and Table XI;* C, *untreated browning soil,* N, *receiving sodium nitrate, and* P, *receiving triple superphosphate.* FIG. 2 *shows the inappreciable effect of the nitrate application and the beneficial effect of the phosphate application.* FIG. 3 *shows the typical yellow first foliage leaves of the diseased plants in the untreated browning soil as compared with the darker green vigorous plants in the phosphated plot.* FIG. 4 *shows the plants in* FIG. 3 *as they appeared two days later. Note that the second foliage leaves in the untreated plot are now yellow, while the first foliage leaves are yellowish brown and drier, and that the phosphated plants are unchanged.*

In Plate I, Figs. 2 to 4, are shown representative pots of Expt. 5 at the time the check plants showed typical browning symptoms. The effects of the fertilizers on the dry weights of these plants (Table XI) are of the same order as in Expts. 1 and 3. The effects on the crown-root systems are quite marked. In Expt. 5, in the diseased soil, the triple superphosphate has more than doubled the number of crown roots, while the sodium nitrate has had no effect; in the healthy soil the phosphate has increased the number by a little over one-quarter, while the nitrate has given no significant increase. Although the phosphate-treated plants in diseased soil showed no disease symptoms above ground (Plate I, Fig. 2, P), yet the increase in the number of crown roots is accompanied by an increase in the number of diseased roots. The higher healthy/diseased (H/D) ratio of these plants and the lower H/D ratio of the nitrate-treated plants over the control, suggest that the phosphate does tend to inhibit the disease, whereas the nitrate tends to augment it. The results in the healthy soil are what might be expected when the original available phosphorus and nitrate nitrogen content are borne in mind.

The outstanding results of Expt. 6 are that the phosphatic and manurial dressings have considerably increased the number and length of crown roots, and that this increase is associated with increased dry weights and greener plants. The phosphated plants usually have a slightly higher, and the manured plants a lower, H/D ratio of crown roots. It is characteristic of the manured plants that the large increase in the numbers of crown roots is concomitant with a large increase in the number of diseased roots; this results in the striking brownish yellow appearance of the roots in mass, which is usually the condition of check plants in diseased soil.

Nitrogenous fertilizers have produced no appreciable effect on crown-root development and consequently no difference in the growth of the plants in diseased soil.

At present it is believed that the H/D ratio of crown roots does not, alone, give an accurate disease index or rating when compared with the above-ground symptoms and dry weights. This will be made clearer by observing the dry weights and H/D ratios of the control and nitrate-treated plants in the diseased soil in Table XI. Similarly, the control in the healthy soil might be expected to have a much higher H/D ratio when compared with the control in the diseased soil. In the meantime, the H/D ratio should be considered only as one of the guides in estimating a disease rating.

It appears from the evidence that the beneficial effects of the phosphate applications and other treatments which widen the P/N ratio, are due primarily to the encouragement of a more vigorous root system and possibly only secondarily to increased resistance of the roots to fungal invasion. A detailed histological and microchemical study of the latter phase is a problem for further research.

Wheat following fallow on these soils does not respond to nitrate applications. This can be explained quite apart from the presence of any soil parasite. Nevertheless, in the browning root-rot problem there are indications that relatively high nitrate associated with relatively low available phosphorus under wheat following fallow is another factor which renders the wheat plants more liable to fungal attack, since where the nitrate is reduced, as in the stubble crop on the same field say, the disease is ordinarily not manifest (*cf.* 46).

Since the application of phosphate to browning soil produces vigorous plants lacking aboveground "browning" symptoms, and increases the yield of grain appreciably, we feel justified in recommending the application of phosphate fertilizers as a control measure for *Pythium* root rot of cereals, even though the question whether the plants are made more disease-escaping or more resistant still remains to be worked out.

The Relation of *Pythium* to the Disease

The results reported in the foregoing sections indicate that unbalanced soil nutrients play an important role in the root-rot complex. The following comparative experiments were planned to determine whether the disease is solely one of mineral deficiency or unbalanced metabolism or is caused primarily by the fungal parasite. In this work no attempt has been made to determine whether the various sterilization treatments produced a stimulating effect on plant growth. It is recognized that total or partial sterilization of soil by steam or by chemicals will produce a stimulation in plant growth due mainly to an increase in available nutrients, and partly to a change in the biological equilibrium of the soil microflora. Waksman (47) has pointed out that "there is no method available for sterilizing the soil without changing its physical and chemical properties". However, Cooke (11), working with Hawaiian soils, found that chloropicrin produced "no purely stimulating effect". The limitations of the soil sterilization method are recognized.

Experiments 7 and 8. In these experiments (Table XII), the respective soil sterilization treatments were carried out in six-inch pots in duplicate 14 days before sowing. The formalin was applied at the rate of ½ gallon of a 2% solution per square foot, and the chloropicrin at 200 lb. per acre. The steam sterilized soil was subjected to 15 lb. pressure for 2 hr. In the preliminary trial (Expt. 7), the formalin approximately doubled the yield, while the phosphate gave about 85% increase. In Expt. 8, the average increase in dry weight for the three sterilization treatments was approximately 80% in the diseased soil and 40% in the healthy, when compared with their respective controls. The healthy soil has given an increase over the diseased soil that is greater than usual for healthy soils. The average dry weight of the three sterilization treatments is the same, namely, 3.925 gm. in the diseased soil and 3.931 gm. in the healthy, though the yield of the diseased control is considerably lower than that of the healthy. It does not seem justifiable, therefore, to base the results of these two sterilization series on chemical stimulation alone, but rather some benefit should be attributed to the removal of the parasitic fungi by sterilization also. Incidentally it appears that steam sterilization has inhibited crown-root development.

TABLE XII

A COMPARISON OF THE EFFECTS OF PHOSPHATE APPLICATION AND STERILIZATION OF BROWNING SOIL IN RELATION TO THE GROWTH OF MARQUIS WHEAT

Treatment	No. of plants	Oven-dry weight, gm.	Crown roots			
			Total no.	% healthy	% diseased	H/D ratio
Experiment 7						
"Diseased" soil:						
Spring, 1934—6 weeks						
None	20	2.434				
Superphosphate	20	4.514				
Formalin	20	4.834				
Experiment 8						
"Diseased" soil:						
Autumn, 1934—7 weeks						
None	20	2.138	9	66.6	33.3	2.00
Superphosphate	20	3.102	38	68.4	31.6	2.17
Formalin	20	3.684	26	100	0.0	
Chloropicrin	20	4.224	25	100	0.0	
Steam	20	3.869	2	100	0.0	
"Healthy" soil:						
None	20	2.828	18	66.6	33.3	2.00
Superphosphate	20	3.332	21	57.1	42.8	1.33
Formalin	20	3.820	20	100	0.0	
Chloropicrin	20	4.428	18	100	0.0	
Steam	20	3.546	3	100	0.0	

In an experiment conducted on drifted top soil and wind eroded soil (subsoil), it was found that the total number of *Pythium*-infested crown roots was greater in the top soil than in the eroded soil, in untreated, phosphated and manured pairs. The untreated top soil gave a better yield than the untreated eroded soil, but the phosphated and manured eroded soil gave actual higher yields of plant growth than the corresponding pairs in top soil. This suggests the probable greater activity of the parasite in the surface soil.

Dickson (13) holds that "the parasite rightly is considered the primary cause of the parasitic disease in plants" when considered in relation to the conditioning or predisposing factors of the environment. From our earlier work on parasitism (46), as well as the results reported here, it appears that species of *Pythium* are the primary cause of browning root rot of wheat, but that unbalanced nutrients obtaining under the summerfallow crop on certain phosphorus deficient soils of Saskatchewan, predispose the seedlings to attack. The effect of the unbalanced nutrients on the parasitic vigor of the fungus is still an unsettled problem.

V. PRELIMINARY PLANT ANALYSES

It has already been pointed out that plants absorb nutrient ions in approximately the proportions in which these ions are present in the nutrient medium (9, 10, 38, 39, 43). Pearsall and Ewing (33), working with radish and turnip principally, found that with abundant nitrates in the external medium there was little protein and relatively high amounts of soluble nitrogen in the plants and, conversely, with low nitrates in the medium there was higher protein nitrogen and lower soluble nitrogen. They state that "An abundant supply of nitrates leads normally to the accumulation of amino-acids, which are formed more rapidly than they are used". It became necessary, therefore, to determine the phosphorus and nitrogen fractions in diseased and healthy plants and compare the results with the available phosphorus and nitrate nitrogen content of the soil areas on which such plants were grown.

The greenhouse material, consisting of both tops and thoroughly washed roots, was oven-dried at 100° C. immediately after harvesting, while in the case of the field material, which consisted of tops only, some sample pairs were dried on the day of collecting and others on the following day, depending on the distance of the field from the laboratory. This method probably had no effect on the phosphorus content, but may have changed appreciably the nitrogen fractions. With the nitrogen determinations, therefore, the greenhouse material should be taken as more indicative of the actual fractions in the growing plants. The total phosphorus, and the total and water-soluble nitrogen were determined by prescribed A.O.A.C. methods (32).* The protein nitrogen was obtained by difference. The phosphorus results are presented in Table XIII, and the nitrogen results in Table XIV.

TABLE XIII

PERCENTAGES OF TOTAL PHOSPHORUS IN DISEASED AND HEALTHY PLANTS, JUNE, 1934

Field	Locality	Phosphorus, %		Diseased as a percentage of healthy
		Diseased	Healthy	
1	(Composite sample)	0.136	0.238	57.1
2	Adanac	0.170	0.273	62.3
3	Paynton	0.155	0.368	42.1
4	Maymont	0.165	0.400	41.2
5	Lac Vert	0.163	0.248	65.7
6	Humboldt	0.139	0.231	60.2
7	Dana	0.153	0.198	77.3
8	Plunkett	0.146	0.226	64.6
9	McLean	0.140	0.267	52.4
10	(Composite sample)	0.154	0.198	77.8
11	(Composite sample)	0.145	0.311	46.6
12	(Composite sample)	0.158	0.292	54.1
	Mean	0.152	0.271	58.45

Of the twelve pairs analyzed (Table XIII), the healthy sample in every case contained a higher percentage of total phosphorus than the diseased sample. The mean of the total phosphorus content of diseased plants considered as a percentage of the healthy is 58.45, which is roughly of the same order as the available phosphorus content of diseased soil considered as a percentage of healthy soil (*cf.* Mean, 1932-33, Table II).

**My thanks are due to J. S. Clayton, M.Sc., of the Cereal Chemistry Laboratory, for making these chemical determinations.*

TABLE XIV

PERCENTAGES OF NITROGEN IN DISEASED AND HEALTHY PLANTS, JUNE, 1934

Field No.	Locality	Nitrogen							
		Diseased				Healthy			
		Total, %	Protein, %	Water-soluble, %	Ratio, P.N./W.S.N.	Total, %	Protein, %	Water-soluble, %	Ratio, P.N./W.S.N.
Greenhouse:									
	Composite	3.54	1.40	2.14	0.65	4.37	2.28	2.09	1.09
	Composite	3.86	1.06	2.80	0.37	4.54	1.63	2.91	0.56
	Mean	3.70	1.23	2.47	0.51	4.45	1.95	2.50	0.82
Field:									
1	(Composite sample)	4.26	2.86	1.40	2.04	4.83	3.64	1.20	3.04
2	Adanac	4.12	2.78	1.34	2.07	4.22	2.96	1.26	2.34
3	Paynton	4.09	2.34	1.75	1.34	4.79	2.78	2.01	1.38
4	Maymont	4.22	2.08	2.14	0.97	5.14	2.79	2.35	1.18
5	Lac Vert	3.58	2.14	1.44	1.47	3.81	2.16	1.66	1.30
6	Humboldt	3.01	1.26	1.75	0.72	3.30	1.77	1.53	1.15
7	Dana	3.31	1.73	1.57	1.10	3.12	1.54	1.57	0.98
8	Plunkett	3.03	2.02	1.01	2.00	3.49	2.19	1.29	1.70
9	McLean	3.36	2.30	1.05	2.18	4.16	2.87	1.28	2.24
10	(Composite sample)	2.62	1.82	0.80	2.28	2.93	1.68	1.25	1.34
11	(Composite sample)	3.56	2.42	1.14	2.12	4.45	3.45	0.99	3.47
12	(Composite sample)	4.01	2.25	1.77	1.27	4.55	2.78	1.77	1.57
	Mean	3.60	2.17	1.43	1.64	4.07	2.55	1.51	1.81

The figures of most interest in Table XIV are the protein nitrogen and water-soluble nitrogen ratios which are lower in the diseased soil than in the healthy in both the greenhouse and field material. That is, there is relatively *more* water-soluble nitrogen and relatively *less* protein nitrogen in diseased than in healthy plants. While these results are decidedly preliminary, they are in general agreement with the findings of Pearsall and Ewing (33). However, it is possible that as more and more of the roots in the diseased areas become infected, normal intake and subsequent utilization of the various nutrients will be greatly disturbed. This may in part account for the generally higher percentage of total nitrogen in healthy plants, though the nitrate nitrogen content of healthy soil is usually lower than that of diseased soil.

Rapid chemical tests for phosphate and nitrate. Wheat seedlings grown in the greenhouse in composite samples of browning and of normal soil collected in early June, 1934, were tested for phosphate and nitrate when the seedlings in the browning soil showed disease symptoms. The methods used were the semi-quantitative rapid chemical tests on plants as described by Thornton and co-workers (44).

Stem portions of diseased seedlings from the browning soil showed a lower content of phosphate and a greater content of nitrate in the sap than healthy seedlings from normal soil. The same relation existed in the roots of diseased and healthy seedlings which were very thoroughly washed and dried between filter paper before testing. Similar results were obtained with maize seedlings. In some fertilizer experiments, applications of either phosphate or nitrate to the soil were readily detected by an increase of these constituents in the sap of the seedlings. The results obtained, though semi-quantitative, were clear cut.

If, as has been suggested (33), with abundant amino acids the cellulose walls become more swollen, and also, as some contend, with high phosphorus the cell walls become harder, then it is reasonable to assume that the unbalanced soil nutrients in certain soil areas lead to a condition in the plants which renders them more liable to the depredations of the parasite. At the same time it must be recognized that a particular balance of nutrients in one soil area may encourage a vigorous vegetative growth in the parasite, in which state it might be most parasitic, whereas in another area conditions may favor sporulation or a relatively quiescent state.

Chlorophyll Content

It is generally conceded that the higher the nitrate content of the soil the greener will be the plants growing thereon (33). Several comparisons were made of the chlorophyll extracts of the tops of healthy and diseased plants from which the lower discolored leaves had been removed. No consistent visible differences could be observed in the respective chlorophyll extracts. It is possible that the damaged root systems of the plants from the diseased areas may have prevented efficient absorption and subsequent utilization of the higher soil nitrate.

General Discussion

In *Pythium* root-rot districts the disease is principally confined to the wheat crop following bare fallow; the stubble crop is rarely sufficiently attacked for aboveground symptoms to appear. The chemical analyses reported in this paper have shown that available phosphorus in the soil is lower and nitrate nitrogen higher in browning areas than in normal areas. It is necessary, therefore, to consider the soil and crop-culture factors, especially the practice of summerfallowing, as they exist in north-central Saskatchewan. The majority of prairie soils, though potentially rich in nutrient elements, respond to certain fertilizer applications (14). Recently a deficiency of available phosphorus in many of these soils has become manifest (14, 29). The replenishment of the nitrate removed by the crops is accomplished by the practice of bare summerfallow cultivation, but the amount of nitrification is closely dependent on seasonal conditions and on frequency of cultivation. In browning root-rot localities and in fertilizer pot trials, no increase in yield of cereals as a result of potash applications has been observed. Potassium is therefore considered to be present in sufficient quantities in Saskatchewan soils where *Pythium* root rot occurs. Wyatt and Newton (51), however, obtained beneficial results from applications of potash on the gray, wooded soils of northern and western Alberta and sometimes also on the black soils of Alberta. The majority of Saskatchewan soils are neutral or slightly alkaline (Tables I and II) with some podsols showing slight acidity, but no evidence of iron or manganese deficiency has been demonstrated as yet. The commoner crop rotations on the prairies are wheat-wheat-summerfallow or wheat-oats-summerfallow, as the majority of farms are devoted almost entirely to grain growing. Mixed farming with grain growing still of major importance is found in the park belt to the north and northeast. This continuous system of grain growing in many of the older districts or on poorer soil in newer districts has apparently reduced the available phosphorus to an amount insufficient for the needs of the crop. Until recently, fertilizer amendments were never applied to replenish the essential elements removed by the crop, and owing to the type of farming, comparatively little farmyard manure has been applied to the land. The foregoing facts, therefore, doubtless account for the low available phosphorus in browning districts, but it is also possible that some of these soils are naturally highly phosphate-fixing, as indicated by the work of Cooke (11), Carpenter (8) and others working on *Pythium* root rot of sugar cane in Hawaii.

The chief purpose of bare summerfallowing on the prairies is to conserve soil moisture for the following crop. Nitrate nitrogen increases because the conditions favor rapid nitrification, and weeds are kept in check by the intermittent cultivation. The lack of positive response of wheat following fallow to nitrogenous fertilizers indicates that the open prairie soils can still produce enough nitrate for the needs of the crop. It should be pointed out, however, that the gray, wooded soils of northern Alberta (51) and northern Manitoba (14) and very likely of northern Saskatchewan also, produce definite increases in yield from nitrogenous fertilizers. Barnes (1) has questioned whether a nitrogen problem really exists in the extensive dry-land farming areas of Western Canada. The moisture hazard appears to be the dominant problem. Now it is possible that in the north-central and northeastern districts where precipitation is usually sufficient for the needs of the crop, excessive cultivation of the summerfallow, performed purposely to control weeds and not necessarily to conserve moisture, may permit the accumulation of nitrate in excess of the demands of the seedlings. They may absorb more nitrate than can be elaborated into reserve nitrogen compounds in a given time. The nitrate may not be unduly excessive, but it may be above the critical level for that element. This condition, if associated with an insufficiency of available phosphorus, may well upset the metabolism of the wheat seedlings at the critical stage of crown-root development and the beginning of the tiller formation. Now well-tilled fallow may contain considerably more nitrate than poor fallow (23); this fact may be of significance in explaining why *Pythium* root rot of wheat is frequently most severe on the best fallows in a browning district. The second or stubble crop of wheat fallowing a browning-diseased crop on summerfallow, does not ordinarily develop browning symptoms. In such a case the nitrate is considerably lower than in the fallow crop, while the available phosphorus has remained practically the same according to available evidence (29). The disease may be prevented from becoming manifest by (*i*) reducing the amount of nitrate nitrogen of many excessively cultivated summerfallow fields in browning districts, as occurs naturally when a stubble crop is grown; and (*ii*) by increasing the amount of available phosphorus by the application of phosphatic fertilizers at the time of sowing the summerfallow crop. Thus it appears that there is a correlation between available phosphorus and nitrate nitrogen and disease expression. It seems also that either "balanced" deficiencies or balanced excesses will inhibit root rot of wheat. Experiments with sugar cane (8, p. 332) indicated that balanced deficiencies did not lead to root rot, but the question of balanced excesses has not been settled.

It is well known that the addition of strawy organic matter to the soil decreases, temporarily, the available nitrate, and our experiments have demonstrated that the addition of cereal straw to browning soil tends to inhibit the disease. This may be due to increased biological antagonism. It is possible, however, that the carbon dioxide evolved by the decomposing organic matter increases the availability of phosphorus. Thus, in Louisiana, both Meyer (28) and Sturgis (42) have shown the importance of increasing or maintaining the availability of phosphorus by the addition of trash to the soil. Meyer considered that as the organic matter is depleted, the soil phosphates as well as those added become less effective. Sturgis found that the increase was most marked in the earlier stages of decomposition. Pittman and Burnham (35) pointed out that in Utah, manuring brings much of the unavailable soil phosphate into action. Now the ploughing under of weeds or other organic matter invariably produces better growth in browning fields. It might reasonably be postulated that the biological activities have reduced the soil nitrate and increased the availability of the soil phosphorus. This may also explain the normal or healthy areas in otherwise browning-diseased fields. The changes in the P/N ratio in the soil under the prevailing crop-culture methods are now more understandable. Our results indicate that an increase in the P/N ratio is accompanied by an inhibition of *Pythium* root rot, while a decrease promotes the disease. The available phosphorus and nitrate nitrogen ratios as shown in Table III, suggest that low available phosphorus is more closely correlated with the disease than high nitrate nitrogen, that is, if the two can be considered separately.

The experimental results of the effects of phosphatic and nitrogenous dressings on *Pythium* root rot in browning and normal soil are in general agreement with the expectations based on the chemical analyses of the soils. Three years' observations of phosphate applications to wheat on fallow in browning districts have shown that the fertilizer has inhibited the root rot generally as indicated by the uniform growth and the absence of patches of brown, dwarfed plants so characteristic of unfertilized check strips. The evenness of the phosphated fields persists until maturity, resulting in uniformity of ripening and plumpness of grain. The effects of various amendments on the crown-root development in browning and normal soil are very illuminating (Table XI). Phosphates and farmyard manure frequently double both the number and the total length of crown roots of wheat in browning soil. The ratio of healthy to diseased roots is not always increased thereby, but "normal" green plants develop (Plate I, Fig. 3, P); this suggests that the more vigorous root systems have allowed the plants to become disease-escaping rather than more resistant. This phase requires further elucidation. Nitrate applications to browning soil usually have no effect on crown-root development; sometimes, however, there is a decrease in number and length as well as a decrease in the healthy/diseased ratio. Our present knowledge of the effects of the soil differences on the parasitic vigor of the fungus is very meagre, and could be discussed only speculatively.

It must not be inferred from the results of the chemical soil analyses and fertilizer experiments that the disease is due merely to nutritional disturbances. In a previous communication (46) it was shown that *Pythium* spp., principally *P. arrhenomanes*, are constantly associated with the lesioned roots of browning seedlings and that they can be readily isolated and made to reproduce the disease artificially. The Canadian strain of *P. arrhenomanes* is vigorously parasitic on wheat seedlings. In this paper some evidence is presented which suggests that the parasite does play an active part.

The plant analyses for total phosphorus and total and water-soluble nitrogen submitted in this paper are only preliminary, although they are in general agreement with the work of others on percentages of phosphorus and nitrogen in plants relative to the amount of these elements in the external medium. The main points ascertained were: firstly, that the total phosphorus was *lower* in diseased than in healthy plants; and secondly, that there is relatively *more* water-soluble nitrogen and relatively *less* protein nitrogen in diseased than in healthy plants. The semi-quantitative rapid chemical tests on diseased and healthy seedlings grown in browning and normal soil in the greenhouse showed a lower content of phosphate and a higher content of nitrate in the sap of diseased than of healthy seedlings. Under the more or less uniform conditions in the greenhouse, no differences in resistance to *Pythium* root rot of the commonly grown wheat varieties have been brought out. Under the more extreme conditions of the field slight varietal differences have shown up in some years, but further field observations are necessary before a definite pronouncement can be made.

The marked similarity between *Pythium* root rot of wheat and *Pythium* root rot of sugar cane is very striking and calls for special comment. Carpenter's recent paper (8), which has just come to hand, presents a capable review of the important findings on the cane disease during the last four years. It appears from the comparative culture studies of Rands and Dopp (36) that the causal organisms in both diseases are geographic strains of *P. arrhenomanes* Drechs. In his earlier work, Carpenter (6, 7) pointed out that excess nitrogenous fertilizers promoted the cane root rot and that the disease incidence varied with the nutrition. More recently, Cooke (11) demonstrated that low available phosphorus on many of the phosphate-fixing soils predisposed the cane to fungal invasion. Heck (17) shortly afterwards considered that *Pythium* root rot of cane was "induced by an unbalanced nutrition in which low phosphorus and high mineral nitrogen are more or less outstanding". This is in agreement with our findings of 1932 and 1933 on *Pythium* root rot of wheat which were reported locally and are published here for the first time. It would appear from Carpenter's last contribution (8) that the phosphate-nitrogen ratio is one of the chief lines of investigation on the cane root rot at present; other unfavorable balances of nutrients and deficiencies of other elements are also being given considerable attention. He pointed out that some varieties of cane are made more susceptible with nitrogen in excess, while other varieties not particularly sensitive to increased nitrogen fall a prey to the parasite under deficiencies of available phosphorus.

In conclusion, our present knowledge of soil factors influencing *Pythium* root rot of wheat indicates that unbalanced available phosphorus and nitrate nitrogen, or perhaps in some instances low available phosphorus alone, is most likely concerned in predisposing the seedlings or rendering them liable to fungal attack.

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A MICROBIOLOGICAL STUDY OF PODSOL SOIL PROFILES

II. LAURENTIAN SOILS[1]

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Abstract

Microbiological studies of samples from the separate horizons of two soils from the Laurentian uplands showed that, as in the case of the soils previously studied from the Appalachian uplands, biological activity was dependent upon the organic-matter relations of the horizons. A_1 (organic-matter) horizons were the most active, as shown by the evolution of carbon dioxide, nitrification of the soil's own nitrogen, by numbers of micro-organisms (bacteria, actinomyces, and fungi) and by the production of ammonia from urea. Bacterial numbers in the illuvial horizons were, however, higher than in the eluvial (leached) horizons above, a condition that did not obtain with the Appalachian soils. The organic matter of a soil bearing a coniferous (spruce) flora was deficient in nitrifying power. The biological activity at different levels of a virgin clay soil was found to be in sharp contrast with that of the podsol soils.

In a recent paper the characteristics of the separate horizons of three heavily leached podsol soils from the Appalachian uplands of Quebec were described (3). The upland podsol soils of the Laurentian plateau are different in several respects from those of the Appalachian chain; their dissimilarity is due mainly to the nature of the parent rock, and less to the climatic environment. A study of two such soils revealed the fact that they contained much more calcium, and more magnesium and potassium than the Appalachian soils, in both the surface soil and the subsoil (6). This suggested that biological activity in horizons of the Laurentian soils might prove to be considerably greater than in horizons of the Appalachian, at least in regard to such activities as have been shown to be to some extent dependent on basic constituents.

This paper is intended to serve as an introduction to the biological characteristics of the separate horizons of podsol soils situated within agricultural districts in the Laurentian plateau, in the Province of Quebec. One soil possessed a cover chiefly of deciduous trees; this soil was selected as reasonably conforming in character with those previously studied from the Appalachian system. A second soil from under a stand of coniferous trees was selected to enable a comparison to be made with the horizons from soil under a stand of deciduous trees, since it has been claimed that the bio-dynamics of such coniferous soils are distinctive, in that nitrification may be adversely affected by an excess of resins, terpenes, and similar compounds, (4, 5); it has also recently been found that a spruce raw humus may be inactive in regard to nitrification (1).

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TABLE I

DESCRIPTION OF SOIL PROFILES

Sample and date collected	Ecology	Depths of horizons sampled, and description
LM June 4, 1933	Mixed coppice of beech, birch, poplar, spruce; secondary growth. 800 ft. above mean sea-level. Lac Mercier, Labelle Co., Que.	A_1, 2 in. black, mucky, spongy, organic matter, sinking into leached layer below. A_2, 3 in. gray sandy loam, blackish at top from superficial horizon. B_1, 4 in. reddish-gray sand with gravel; infiltration from A_2 horizon; red streaks. B_2, 3 in. red gravelly sand, extending to below 12 in., where subsoil is richer in iron.
VC June 8, 1933	Regenerated spruce forest. 500 ft. above mean sea level. Val St. Michel, Quebec Co., Que.	(A_0, not collected; spruce needles, decomposing with gray fungus flora). A_1, 2 in. dark, fibrous, spongy, semi-decomposed organic matter, with fibrous root material. A_2, 4 in. gray sand. B, 6 in. red-brown sand, extending to below 12 in., with "ortstein" concretions fragmenting by hand pressure; organic matter and fibrous roots present.

In order to compare the biodynamics of a representative soil of the type that is not affected by climate to such an extent, samples were also taken from a virgin clay soil, at 3-inch intervals down to a depth of one foot. No chemical analyses were made with these subsamples. The separate horizons were sampled in the manner previously described (3).

The analytical methods used were those described in the previous study. In addition, analyses were made by the Department of Chemistry, Macdonald College, for certain physical and chemical factors, as shown in Tables II and III.

TABLE II

PHYSICAL CHARACTERISTICS OF SAMPLES FROM SOIL HORIZONS

Soil	Horizon	Moisture in fresh soil, %	Hygroscopic moisture, %	Loss on ignition, %	Water-holding capacity, % of air dried soil
LM	A_1	73.28	7.21	47.46	165.6
	A_2	16.81	0.28	1.60	26.0
	B_1	14.09	0.22	1.02	23.1
	B_2	19.02	0.63	2.40	24.9
VC	A_1	49.42	6.15	50.78	217.6
	A_2	20.36	0.23	1.44	31.5
	B	43.01	9.37	32.70	59.0

TABLE III

CHEMICAL CHARACTERISTICS OF SAMPLES FROM SOIL HORIZONS

Soil	Horizon	Organic carbon	Total nitrogen	Ratio C/N	CaO	MgO	P_2O_5
LM	A_1	22.65	1.018	22.25	1.43	0.46	0.13
	A_2	0.88	0.051	17.26	2.05	0.82	0.06
	B_1	0.36	0.027	13.36	2.19	0.90	0.12
	B_2	0.88	0.060	14.67	2.23	0.87	0.17
VC	A_1	25.47	0.861	29.58	0.78	0.26	0.14
	A_2	0.72	0.049	14.69	0.99	0.19	0.06
	B	9.80	0.167	58.68	0.91	0.35	0.12

The biological determinations made were those for the evolution of carbon dioxide, for the estimation of bacteria and actinomyces on Thornton's medium, and of fungi on a dextrose agar of pH 4.0, for nitrification of the soil's own nitrogen, and for the production of ammonia from urea. The results are shown in Tables IV to VI, and clearly indicate an activity similar in most respects to that found in the separate horizons of the Appalachian soils.

The biological differences between the organic matter horizons and the leached horizons were very similar to those in the Appalachian upland soils; the average proportional level of activity, as represented by the evolution of carbon dioxide, in the leached horizons was 3.7% of that in the organic matter horizons (Table IV). In regard to bacteria and actinomyces, a proportional value of 5.9% was obtained for both groups of organisms. It is of interest to note that the amounts of carbon and nitrogen in the leached horizons were, on the average, 3.4 and 5.0% respectively of the carbon and nitrogen in the organic matter horizons.

TABLE IV

BIOLOGICAL CHARACTERISTICS OF SAMPLES FROM SOIL HORIZONS (ON BASIS OF OVEN DRIED SOIL)

Soil	Horizon	Carbon dioxide, mg. per 100 gm. per 100 hr.	Bacteria per gm.	Actinomyces per gm.	Fungi per gm.
LM	A_1	211.60	9,792,000	1,104,000	190,700
	A_2	7.85	369,000	53,300	10,280
	B_1	2.78	399,900	(1,000)	(1,000)
	B_2	4.52	1,006,000	(1,000)	(1,000)
VC	A_1	278.10	8,209,000	446,000	487,000
	A_2	14.06	550,000	30,830	2,690
	B	17.56	593,000	12,370	5,580

The numbers of bacteria in the illuvial horizons of both Laurentian profiles were slightly higher than they were in the leached horizons; this can be contrasted with the condition of the same horizons of the Appalachian soils. The same distinction cannot be made with regard to the actinomyces, for in all of the soils so far examined, their numbers were least in the illuvial horizons.

An outstanding difference will be noted in Table V, in respect of nitrification in the "coniferous" soil (VC); no nitrates were formed in the eluvial horizons A_1 and A_2, though nitrogen in these was not considerably less than in the corresponding horizons of the Laurentian soil with a "deciduous" cover. Reference to Table III will show that horizon B of soil VC, in which nitrates were formed to a greater extent than in any other horizon except A_1 of soil LM, contains a high proportion of organic carbon as well as nitrogen. From this it seems possible to conclude that there was less of any inhibitory substances such as may have been present in the two superficial horizons, or that the nitrogen compounds present in the illuvial horizon were different from those in the eluvial horizons. The organic matter present, as both carbon and nitrogen, clearly affected the moisture conditions in this lowest horizon, as shown in Tables I and II. The organic carbon clearly did not affect the evolution of carbon dioxide, nor were the numbers of micro-organisms dependent upon it, in comparison with the other horizons. The horizon under discussion contains, also, a lower percentage of bases and of phosphoric acid than those in which nitrification was not so well developed. In view of the prevalence of coniferous soils, it would appear necessary to make a closer study of the conditions that affect the activity of the nitrifying bacteria, since many such soils, after cultivation, prove to be relatively infertile and have a low capacity for nitrification (4).

TABLE V

NITRIFICATION IN SAMPLES FROM SOIL HORIZONS

(Nitrate nitrogen, parts per million, oven dried soil)

		5 days	51 days	104 days	317 days
LM	A_1	trace	275.10	453.90	599.20
	A_2	nil	10.78	19.11	27.60
	B_1	nil	nil	8.56	10.39
	B_2	nil	nil	9.95	10.18
			47 days	100 days	313 days
VC	A_1	—	nil	nil	nil
	A_2	—	nil	5.6	trace
	B	—	14.76	21.94	29.72

TABLE VI

PRODUCTION OF AMMONIA FROM UREA, BY SAMPLES FROM SOIL HORIZONS

(Mg. NH_3 nitrogen per cc. of culture)

		24 hr.	53 hr.	72 hr.	120 hr.	168 hr.	11 days	20 days	40 days
A. Deciduous podsol soil									
LM	A_1	0.57	10.17	17.63	23.29	22.80	23.26	23.17	19.97
	A_2	0.20	2.27	3.84	7.69	9.84	12.66	13.19	12.46
	B_1	0.08	0.47	1.76	5.25	6.97	9.23	11.75	8.81
	B_2	0.05	0.97	2.02	4.73	7.72	9.82	10.06	8.88
B. Coniferous podsol soil									
VC	A_1	0.27	6.11	17.66	24.15	25.14	22.42	22.92	20.98
	A_2	0.13	1.01	2.17	5.59	8.14	11.16	11.87	10.62
	B	0.07	0.20	1.34	6.77	10.54	15.19	16.12	14.27

NOTE:—*To each 50 cc. of 5% urea solution, containing 0.05%* K_2HPO_4, *was added a mass of moist soil equivalent to 5 gm. of oven-dried soil.*

In the test for the decomposition of urea, the maximum amount of ammonia was produced by the organic-matter horizon of the "deciduous" soil after 5 days' incubation (See Table VI). In none of the other horizons was the limit of decomposition attained, even after 40 days' incubation. It is of interest to note that the illuvial horizon of the "coniferous" soil, having a high percentage of carbon, produced more ammonia than the eluvial (leached) horizon; in this experiment, however, as in that reported in the previous study, the ammonia produced was not proportional to the amount of organic carbon.

The results obtained from the separate layers of the clay soil show clearly the difference between the two types of soil. The values for moisture, evolution of carbon dioxide, and for bacteria are shown in Table VII.

TABLE VII

BIOLOGICAL ACTIVITY IN SAMPLES FROM DIFFERENT DEPTHS OF A VIRGIN CLAY SOIL

Depth of sample, in.	Moisture, %	Carbon dioxide mg. per 100 gm. per 100 hr.	Bacteria per gm. dry soil
0— 3	26.89	17.50	15,540,000
3— 6	20.98	8.55	7,404,000
6— 9	21.40	5.28	9,721,000
9—12	24.10	3.29	6,561,000

Conditions similar to those in the clay soil are probably prevalent in most soils in which the organic matter is evenly distributed vertically to some depth. Brown and Benton (2) report bacterial numbers of the horizons of several agricultural soils of Iowa; they include one virgin soil under grass, in which there was a sharp decline between horizons, but the decline occurred well below the usual limit of ploughing.

Acknowledgment

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A MICROBIOLOGICAL STUDY OF PODSOL SOIL PROFILES

III. BACTERIA FOUND IN SEPARATE HORIZONS[1]

By P. H. H. Gray[2]

Abstract

An investigation has been made to determine the presence of bacteria concerned in certain important biochemical reactions in the separate horizons of five heavily leached (podsol) soils of Quebec. Cellulose decomposing bacteria were represented by a species of *Micrococcus*, and by *Cytophaga hutchinsoni*. Organisms of the group *Mycobacteriaceae* were found in mediums devised to isolate bacteria able to decompose phenol and naphthalene. Of the nitrogen-fixing bacteria the aerobic organism *Azotobacter* was absent, but the anaerobic organism, *Bacillus amylobacter*, was present in all of the horizons. The decomposition of urea was due to Gram-negative spore-forming rods whose systematic position has not been determined.

Podsol soils owe their peculiar characteristics to the fact that under the climatic conditions in which they are most commonly found a large part of the organic residues in the surface horizons remains only partially decomposed. Such decomposition as occurs gives rise to considerable acidity, that removes from the mineral part of the soil below a large proportion of the available bases.

The biological problems presented by the well defined horizons of podsol soil profiles appear to have important bearings in relation to the ecological aspects of forestry and agriculture. The conditions that encourage a certain type of forest cover will affect the value of the soil in agriculture. This is due largely to the nature of the residues from the forest cover, and their ultimate decomposition or resistance to the action of soil micro-organisms. From the aspect of microbial nutrition with especial reference to sources of carbon, it would appear that the important fractions of the plant residues consist in (a) those soluble in water, (b) the more resistant materials, including cellulose, lignin, and protein compounds. Among the compounds commonly associated as lignin, or the lignin-humus complex, are phenolic and other compounds with a ring structure; similar compounds may be present in the protein fractions. These fractions have been found to differ according to the nature of the cover, but studies have not as yet been made to determine the specific sources of biological activity; it is therefore impossible at present to state that one group or another of micro-organisms is of the greater importance in the transformation of the residues.

A number of studies have been made on the nature of the biochemical activity of the undecomposed and semi-decomposed horizons of the organic mat overlying the soil, chiefly in relation to silviculture, and the presence of mycorrhiza and other fungi reported. The formation of ammonia and of nitrate nitrogen in forest soils has also received attention, and some studies have been made on the bacterial flora of forest soils under different climatic conditions. In regard to podsol soils, Rasumov and Remesov (4) have determined the morphology and number of organisms, by a direct method, in separate horizons of soil profiles. No studies have yet been made as to the nature of the bacterial flora concerned in processes of decomposition in the separate horizons of soils representative of the podsol group.

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[2] *Assistant Professor of Bacteriology and Head of the Department of Bacteriology, Macdonald College.*

The present paper reports a preliminary survey of certain groups of bacteria, isolated from the separate horizons of a few heavily leached podsol soils of Quebec, certain characteristics of which have been described in previous papers (1, 2).

The soils from which isolations were attempted have been designated by the letters used previously. In addition, isolations were attempted from the horizons of a heavily leached lowland podsol soil, under mixed coppice (deciduous) near St. Michel de Rougement, Rouville County, Que.; this soil, sampled on June 20, 1933, has been designated as MR.

In regard to the organisms isolated from any horizon, it must be recognized that the presence of any organism in the lower layers (horizons) of soil may be due solely to the movement of water. This may be particularly important in relation to the bacteria utilizing soluble material derived from the organic matter. It must also be recognized that it is not possible to obtain a sample from one horizon without some admixture of insoluble material from a contiguous horizon. The survival of an organism may, however, be dependent upon the conditions in the soil material, the bulk of which gives an environment similar to natural conditions. The samples were kept in closed containers that did not permit excessive loss of moisture; the conditions in the samples thus resembled the natural condition, so that the organisms isolated would probably prove to be those capable of active life in the soil.

In all of the isolations that were attempted, with the exception of those for bacteria decomposing urea, the same basic mineral medium was used, to which were added sources of carbon or nitrogen as required. The composition of the mineral salts medium was as follows:

K_2HPO_4 0.1%; $MgSO_4.7H_2O$ 0.01%; NaCl 0.02%; $CaCl_2$ 0.01%; $FeCl_3.6H_2O$ 0.002%.

A search was made for bacteria belonging to the following groups that are of biochemical importance in agriculture.

1. Bacteria decomposing cellulose.
2. Bacteria decomposing benzene-ring compounds.
3. Nitrogen-fixing bacteria.
4. Bacteria decomposing urea.

Cellulose Decomposition

Soils of 1931

Two grams of moist soil from each horizon of soils R and S were added to 25 ml. of mineral salts medium in Erlenmeyer flasks of 125 ml. capacity, in three series; to one was added 0.2% ammonium sulphate, to another was added 0.2% sodium nitrate; to the third no nitrogen was added. A sterile filter paper, Whatman No. 41, 11.5 cm. in diameter, was folded to admit the wide end into a flask, so that it remained upright in the medium, and thus provided a large area of surface for aerobic organisms. On a basis of dry soil, the amounts of inoculum were in most cases more than one gram. The cultures were incubated at 26° C. on July 12, 1932. After 17 days the cultures were examined; with one exception, the organisms found on the cellulose were fungi.

The inoculum from horizon B of soil R developed a growth of *Cytophaga hutchinsoni*, together with associated bacteria, in the medium containing sodium nitrate. The results are shown in Table I.

TABLE I

ORGANISMS DEVELOPING IN CELLULOSE MEDIUMS
(Soils of 1931)

Soil	Horizon	Nitrogen added to medium		
			Sodium nitrate	Amonium sulphate
R	A_1	Fungus	Fungus	Fungi
	A_2	No growth	Fungus	Fungus
	B	No growth	*Cytophaga et al.*	Fungus
S	A_1	Fungus	Fungi	Fungi
	A_2	No growth	No growth	Fungi
	B	No growth	No growth	Fungus

Conditions in these horizons were evidently unsuited, with the exception noted, for the viability of bacteria commonly found in agricultural soils and capable of growth in the mediums used. It is possible that the addition of the mass of soil, from 0.62 to 1.7 gm. on the oven-dry basis, so altered the conditions in a medium as to render it unsuitable for the bacteria.

Soils of 1933

Approximately 1-gm. samples of fresh moist soil from the separate horizons of soils LM, VC and MR, were inoculated into cellulose-salts medium (June, 1933); in the case of the organic matter horizons, 1 : 10 dilutions were also inoculated. Cultures were examined at intervals for a month, and cellulose-destroying bacteria were found in two organic matter horizons (A_1) and in one illuvial horizon (B_2). The results are shown in Table II.

The bacterial decomposition was wholly that of the organisms mentioned; no *Vibrio* spp. were noted, whose characteristic action is usually shown by disintegration of the fibres of the filter paper. The other forms noted as present were usually short rods, commonly found as contaminants in *Cytophaga* cultures.

TABLE II

Organisms developing in cellulose mediums

(Soils of 1933)

Soil	Horizon	Organisms present after		Remarks
		15 days	30 days	
LM	A_1	No growth	Black slimy growth	No fungi; no bacteria isolated.
	A_1 (1 : 10)	*Micrococcus*	*Micrococcus*	Not isolated.
	A_2	No growth	No growth	—
	B_1	No growth	No growth	—
	B_2	*Cytophaga*	*Cytophaga*	Other forms present.
VC	A_1	Yellow growth	Fungi; black growth	No *Cytophaga*.
	A_1 (1 : 10)	No growth	Slimy	—
	A_2	No growth	Gray and brown fungi	—
	B	No growth	Fungus	—
MR	A_1	*Cytophaga*	—	—
	A_1 (1 : 10)	Yellow growth and fungi	—	Not isolated.
	A_2	Yellow growth	—	*Cytophaga* and other forms.
	B	No growth	—	—

In a later test (October 31, 1933) small quantities of soil from horizons of VC and MR were inoculated into test tubes of medium, cellulose being added in the form of a strip of filter paper. *Cytophaga sp.* was found again in the organic matter horizon of soil MR, but did not develop from soil VC. The organic matter (A_1) horizon of the latter soil consisted of spruce raw humus. The pH of the samples, after storage, varied from 4.00 to 4.67.

Bacteria Decomposing Benzene-ring Compounds

Soil from the horizons of the lowland podsol MR, and of the "coniferous" podsol, VC, was inoculated into a sterile medium composed of mineral salts, ammonium sulphate, and either phenol, 0.05%, or naphthalene crystals previously sterilized in alcohol. The soils had been stored for four months in a closed container before the cultures were made. In these tests, positive proof of an organism's capacity to decompose the carbon compound supplied was accepted only after growth had become well developed in sub-cultures.

When phenol was used in the selective medium, cultures belonging to the genus *Mycobacterium* were isolated from the organic matter horizon and from the B horizon of the lowland podsol soil MR. In pure culture these organisms attacked phenol, but not naphthalene. The cultures from the horizons of the "coniferous" Laurentian podsol VC proved sterile. When naphthalene was used in the selective medium, organisms of the genus *Mycobacterium* were found to be present in all of the horizons of soil VC, and in the B horizon of soil MR. The mycobacteria isolated from the B horizons, after transfer and plating in beef-peptone agar, were found capable of attacking phenol, but not naphthalene; those isolated from the eluvial (A_1, A_2) horizons of the "coniferous" soil attacked naphthalene and phenol, but appeared later to have lost the ability to decompose the former compound.

The bacteria were classified as belonging to the genus *Mycobacterium* on the basis of their resemblance in morphology, and in growth on nutrient media, to similar organisms previously isolated by means of selective media containing the same sources of carbon (3).

Nitrogen-fixing Bacteria

Attempts were made to isolate aerobic bacteria by means of nitrogen-free mediums containing mannitol or dextrine, and anaerobic bacteria with the same basic medium containing dextrose.

Soils of 1931

About 1 gm. of air-dried soil from each horizon of soils R and S was added (March 8, 1932) to 20 ml. of medium in Erlenmeyer flasks of 125 ml. capacity; the medium contained 1% of mannitol or of dextrine, and 0.5% of calcium carbonate. The cultures were incubated at 27° C. After nine days gas was visible in all of the cultures, and an examination of films showed that *Azotobacter* was absent from all. This was confirmed by plating on dextrine agar. Three months later the soils were inoculated again into mannitol medium, and *Azotobacter* again found to be absent. A summary of the results of this later test is given in Table III.

TABLE III

MICRO-ORGANISMS FOUND IN NITROGEN-FREE MANNITOL CULTURES

(Samples from soil horizons of 1931)

Soil	Horizon	Gas	Organisms after 5 days	Cultural character after 17 days
R	A_1	Present	Short rods, long rods, spores.	Moulds
	A_2	Present	Long rods	Moulds
	B	Present	Cocci, spores	No change.
S	A_1	Present	Long rods, spores, *B. amylobacter*	Moulds.
	A_2	None	Long rods	No gas.
	B	Present	Cocci, rods, spores	No change.

Soils of 1933

One-gram samples from the separate horizons of soils LM, VC, and MR were added (June, 1933) to mannitol medium. *Azotobacter* was not found in any of these horizons; the presence of gas indicated the prevalence of anaerobic bacteria. Cells resembling the organism *Bacillus amylobacter*, and other forms, were found by direct observation of wet-mount films. An attempt was made, therefore, to determine the morphology of the anaerobic nitrogen-fixing bacteria in the horizons of the soils sampled in 1933. For this purpose a medium containing 1% of dextrose was prepared in test tubes, and cultures prepared anaerobically by means of platinized asbestos. The mineral-salts medium in double strength and 2% dextrose solution were sterilized separately and mixed just before inoculation with a small mass of soil. Before incubation, the test tubes containing the inoculum were heated to 80° C. for 10 min. They were then cooled rapidly and the mouth closed with the rubber stopper bearing the heated platinized asbestos; hydrogen was passed through for two minutes, and all connections sealed with gold size.

After a short time of incubation at 20° C., cultures from all of the horizons developed gas, a cloudy white growth becoming a viscid or a granular sediment, and an odor of butyric acid. Microscopic observation showed that organisms, referable to the morphological type *Bacillus amylobacter*, were present in large numbers, the spores characteristically swelling the cells or situated terminally. After transference to fresh medium the same characteristic growths developed, and in slanted agar of the same composition clostridium forms were found after staining. The isolation of a pure culture was not attempted, except in the case of the culture from horizon B_1 of soil LM, for the combination of heating with the selective medium allowed but few contaminants to grow.

Bacteria Decomposing Urea

The selective medium used for the development of urea-decomposing bacteria consisted of urea 2%, dipotassium hydrogen phosphate 0.05%, in distilled water. Small masses, less than 1 gm., of material from each horizon of soils LM, VC and MR were added to the medium, and incubated at 26° C. (October 28, 1933).

The presence of urea-decomposing bacteria was indicated by a strong odor of free ammonia. Transfers were then made to test tubes of urea medium, containing 5% beef extract as a source of carbon. After incubation for a few days at 26° C., the presence of ammonia was confirmed by testing with Nessler's reagent in a porcelain plate; cultures that gave a positive test were plated on urea beef-extract gelatine. Bacteria able to split urea in pure culture were isolated from all horizons of the two Laurentian soils, LM and VC, but not from the lowland soil MR. The bacteria were found to be Gram-negative spore-forming rods, growing well on urea beef-extract gelatine, but poorly on beef-peptone agar. Further studies are needed to determine their systematic position.

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EFFECT OF FROST ON WHEAT AT PROGRESSIVE STAGES OF MATURITY

III. MILLING AND BAKING QUALITY[1]

BY R. NEWTON[2] AND A. G. MCCALLA[3]

Abstract

The yield of flour from unfrozen samples of wheat cut at progressive stages of maturity increased until the dry matter of the grain at the time of cutting reached 58%, and thereafter remained constant. Frost exposure reduced the flour yield at all stages of maturity, the reduction being roughly proportional to the immaturity of the sample and the severity of the exposure. The yield from mature, frozen samples was slightly but definitely lower than that from comparable unfrozen checks.

The baking quality of the unfrozen checks was relatively high, even when the wheat was cut while immature. Frost exposure reduced the quality of immature samples in proportion to the immaturity of the grain and the severity of the exposure, but had little if any effect on mature samples. Flour from immature, frozen samples deteriorated in storage more rapidly than did that from the unfrozen checks.

Reduction in flour yield was proportional to reduction in grade but reduction in baking quality was, on the average, less than anticipated from the grading results.

The earlier conclusion is confirmed that 58% dry matter represents a critical stage in the development of wheat, and that all samples harvested after this stage can be considered mature.

Introduction

Continuing the series of papers in which were described the physical characteristics of wheat kernels from plants which had been subjected to graded frost exposures at progressive stages of maturity in the seasons of 1929, 1930 and 1932 (7), and the composition and certain biochemical properties of the grain and flour from the 1930 samples (6), we now present the results of milling and baking tests of the 1930 samples.

A full description of the origin and treatment of the samples will be found in the first paper (7). They were all of the Marquis variety.

The 1929 samples, of four varieties, were also milled and baked experimentally, but notwithstanding that the frost exposures had been sufficient to cause in several instances a substantial lowering of the grade, as described in the first paper, no significant differences in flour yield or quality could be demonstrated. Laboratory milling by the equipment and methods so far available is admittedly an inadequate test for differences in milling quality that may be of real significance in commercial practice, and it seems likely that such differences actually existed, at least in those cases in which blistering was severe enough to reduce the weight per bushel (7). Laboratory baking methods and technique have, however, progressed to a point where we may with some confidence expect to detect any important differences in baking quality. The absence of demonstrable differences in the 1929 samples therefore suggests that even in those cases where the frost exposure led to a heavy cut in grade, the injury was little more than skin deep.

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Profiting by this experience, we made the 1930 exposures more severe, and in the resulting samples it was possible to show chemical differences (6) and differences in both milling yield and baking quality. In addition to the results on the latter points, some data on the storage properties of flour from frosted wheat are included in this paper.

Previous Work

Although there have been numerous investigations on the effect of frost on the composition of wheat, there have been few which have included direct estimates of milling and baking quality.

References to the milling and baking value of frosted wheat are made in a number of the reports of the Dominion Grain Research Laboratory, two of which will be cited. In the first (2), it was reported that the most noticeable effects of heavy frost on the baking quality of flour milled from frosted wheat were a reduction in loaf volume and the production of bread which was gray or dark in crumb color. Results of individual tests indicated that when wheat was affected by only a slight bran frost, the quality was practically unimpaired. In the second report (3), it was stated that the frosted wheat samples of the 1929 crop studied had apparently been exposed to frost when they were nearly mature. This was advanced as the reason why the quality was not injured to the extent suggested by the outward appearance of the kernels.

Results obtained by Whitcomb, Day and Blish (8) indicated a higher value for frosted wheat than that with which it is usually credited, but did not warrant definite conclusions.

Whitcomb and Sharp (9) made a study of wheats from heads harvested at various stages of maturity, part of each collection being frozen artificially. On account of difficulties in milling which they point out, they drew no conclusion with regard to the effect of the frost on the flour yield of immature samples. They found no differences attributable to frost in the flour yields of relatively mature samples. The loaf volume was smaller and the color and texture of bread much poorer, when made from frosted, immature wheat than when made from the unfrozen checks, but these differences disappeared in samples which had been frozen while containing less than 46% moisture.

Johnson and Whitcomb (5) studied Marquis wheat frozen to varying degrees at different stages of maturity. They found that temperatures from 29° to 27° F. were low enough to cause damage to immature wheat. The stage of maturity and severity of the frost determined the extent of the damage to baking quality. It was concluded that if wheat were frozen after the moisture content had diminished to 46 to 44%, *i.e.*, at or after the stiff dough stage, it was capable of producing as good bread as unfrozen wheat harvested at the same stage.

Geddes, Malloch and Larmour (4) reported the results of an extensive co-operative study of 228 samples of frozen and unfrozen wheat. The heavily frosted samples were harder to mill than the unfrozen or lightly frosted samples, owing to the tough and fibrous nature of the middlings. Flour yield decreased with grade. The percentages of bran frosted, heavily frosted and immature kernels were negatively correlated with flour yield. Baking quality was determined in three laboratories, using either a 55% patent or a straight grade flour, and four baking formulas. Absorption was higher in the lower grades. The average baking quality as measured by loaf volume, crumb color and texture decreased with grade, except in the instance of No. 4 which was superior to No. 3 Northern. Partial correlations showed that in estimating baking quality the various forms of damage need not be considered individually. On the whole, the grades given by official inspectors were logically related to damage found in this investigation. The anomalous relation found between No. 3 Northern and No. 4, the authors believe, would not be likely to recur, as the 1930 revisions of the Canada Grain Act were in line with their findings.

Milling Quality

INDIVIDUAL SAMPLES

The individual samples of wheat described in the first paper of this series (7) were milled on an experimental mill, a straight grade flour (4) being produced. The results for all samples, expressed as percentages of the wheat, are presented in Table I, and those for the samples grown at the south end of the field

TABLE I

FLOUR YIELDS OF INDIVIDUAL SAMPLES

Dry matter at cutting, %	Straight flour, % of wheat			Dry matter at cutting, %	Straight flour, % of wheat		
	8° frost	4° frost	Check		14° frost	10° frost	Check
31.2	40.7	47.7	46.1	34.0	36.1	38.4	52.5
34.9	44.5	52.7	53.4	38.1	39.7	40.8	57.9
39.4	49.9	57.4	59.6	40.9	35.9	36.3	58.5
43.8	44.2	57.7	62.2	45.5	43.2	43.3	63.8
46.8	53.2	61.4	64.6	46.7	48.0	47.2	65.4
48.3*	53.2	63.2	67.4	50.5*	50.6	50.6	65.5
50.5*	50.6	54.7	66.0	51.1*	49.2	—	62.7
51.1*	47.6	—	62.7	53.3*	50.6	50.2	66.4
51.3*	48.6	56.4	65.8	54.9	60.4	60.6	68.6
55.8*	51.0	56.0	68.6	57.4*	54.2	53.8	68.5
56.8	61.8	68.9	70.6	58.1*	61.4	—	69.2
57.3	65.1	68.4	69.8	58.5	66.3	65.8	70.0
58.1*	63.4	—	69.2	59.1*	61.6	—	68.0
59.1*	61.8	—	68.0	65.5*	66.0	—	67.9
65.5*	70.4	—	67.9	65.6	66.7	—	69.6
65.6	64.9	—	69.6	69.4	—	—	68.9

**Samples from north end of field.*

expressed as percentages of both the wheat and the total flour in Fig. 1. The results for south end samples only are included in these graphs because the other samples varied considerably in flour yields owing to the uneven ripening discussed in the first paper.

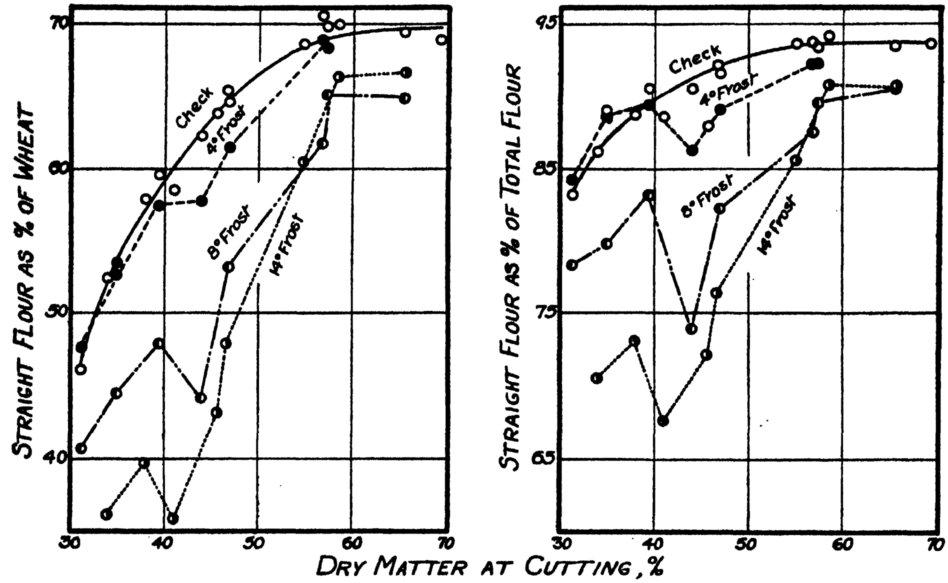

FIG. 1. *Flour yields of "south end" individual samples, subjected to various intensities of frost, in relation to maturity at cutting.*

The yield of straight flour from the unfrozen checks rose from 46 to about 69% of the wheat as the grain matured, until the dry matter content of the grain at cutting reached 56 to 58%. There were no significant differences among the later samples. The straight grade flour, calculated as a percentage of the total, increased with progressive maturity, and also reached a maximum in those samples containing 58% or more dry matter at the time of cutting.

In general, the flour yield from samples exposed to four degrees of frost was only slightly less than that from the comparable checks. The north end samples, which show quite heavy reductions as a result of this light frost, must be treated as a special case, because of their considerable admixture of green kernels. There were no differences in the two most immature samples, probably because immaturity caused almost as much shrivelling as was caused by the combined effect of immaturity and light frost. No mature samples were exposed to four degrees, but the two latest collections in this group (56.8 and 57.3% dry matter) were slightly reduced in flour yield, the blistering of the kernels apparently being sufficient to affect the milling quality appreciably.

Exposure to eight degrees of frost reduced the flour yield from immature samples markedly, and affected even the mature samples. Not only was there a reduction in the yield of straight flour, but there was also a larger proportion of the total discarded as "feed". The yields reached a fairly constant level for all samples cut after the dry matter content reached 58%, but the level was approximately 4% lower than that reached by the unfrozen checks.

Exposure to 10 and 14 degrees of frost resulted in even more marked reduction in flour yields from the immature samples, but there were only slight differences between the results for comparable samples exposed to these two temperatures. There were no significant differences in the effects of the different exposures on mature wheat.

The difficulty encountered by Geddes, Malloch and Larmour (4) in reducing the middlings of low grade wheats was met with in milling the severely frozen samples of this series. The time required to mill a sample of the severely frozen wheat was considerably longer than that required to mill the unfrozen checks. Immature samples were more difficult to mill than mature ones, but immaturity was a less important factor than severe frost treatment.

COMPOSITE SAMPLES

The material remaining after the individual samples had been studied was made up into composite samples as follows: Equal amounts of each of the five most immature check samples were thoroughly mixed and the mixture designated C1. Those samples exposed to four degrees of frost and falling within the first five collections were combined in equal quantities and this mixture designated C2. Similarly the samples harvested at the same stages and exposed to 8, 10 and 14 degrees of frost were composited and designated C3, 4 and 5 respectively. Similar composites were made up of the other material, the samples from each exposure and from the two ends of the field being kept separate. The complete series consisted of 31 samples made up as indicated in the first columns of Table II.

The grades of the individual samples have already been reported in the first paper of this series (7). The composites were graded independently and the results, which agree well with those for the individual samples, are included in Table II. These are referred to again in the general discussion at the end of this paper.

Portions of each of the composites were milled at the Universities of Alberta, Manitoba and Saskatchewan. The averages of the flour yields obtained at the three places are presented in the last columns of Table II, and those for the samples grown at the south end of the field in Fig. 2.

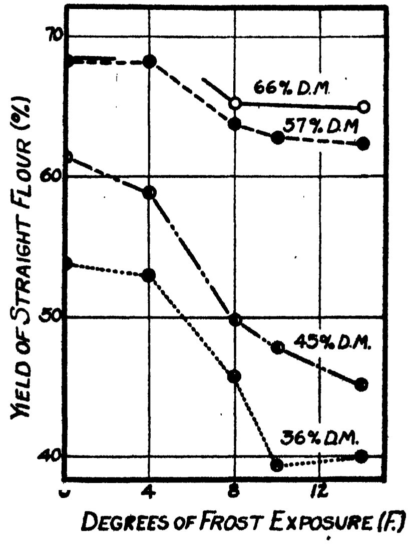

FIG. 2. *Flour yields of "south end" composites, of four stages of maturity, in relation to frost exposure.*

TABLE II

FLOUR YIELDS OF COMPOSITES

Sample No.	No. of individuals composited	Av. dry matter at cutting, %	Degrees of frost, F.	Grade**	Straight flour	
					% of wheat	% of total flour
C1	5	35.5	0	F	53.8	85.0
2	3	35.2	4	F	53.1	86.0
3	3	35.2	8	F	45.7	80.5
4	2	36.0	10	F	39.4	69.2
5	2	36.0	14	F	40.0	70.8
6	5	44.8	0	4	61.5	89.2
7	2	45.4	4	5	59.1	85.8
8	2	45.4	8	F	49.8	77.3
9	3	44.3	10	F	47.7	76.4
10	3	44.3	14	F	45.1	73.1
11*	6	50.8	0	5	64.0	88.6
12*	3	50.0	4	6	59.1	85.0
13*	4	50.3	8	F	47.0	75.6
14*	2	51.9	10	F	50.7	76.6
15*	3	51.6	14	F	50.7	76.5
16	4	56.3	0	2	68.3	91.2
17	2	57.0	4	4	68.3	90.4
18	2	57.0	8	5	63.8	87.1
19	2	56.7	10	6	62.9	86.4
20	2	56.7	14	6	62.3	86.1
21*	4	57.6	0	2	67.3	90.3
22*	1	55.8	4	F	56.8	81.6
23*	3	57.7	8	6	61.9	85.7
24*	1	57.4	10	F	56.8	81.3
25*	3	58.2	14	6	57.9	82.6
26	2	67.5	0	1	68.2	90.8
27	1	65.6	8	5	65.2	88.9
28	1	65.6	14	5	64.9	89.2
29*	1	65.5	0	2	68.4	91.2
30*	1	65.5	8	3	69.2	91.0
31*	1	65.5	14	6	66.8	89.2

**Samples from north end of field.*
***F = Feed.*

These results are in good agreement with those obtained with the individual samples. The yield of flour from the unfrozen checks reached a maximum for those samples cut at an average dry matter content of 57%. Four degrees of frost reduced the yield slightly, while 8, 10 and 14 degrees reduced it markedly except in the more mature samples. In all but one case (Sample C13) eight-degree exposures reduced the yield less than did exposures of 10 and 14 degrees. The yields from severely frozen samples cut when the average dry matter content was 57% were lower than those from the most mature, frozen samples. This result is not in disagreement with the conclusion reached as a result of study of the individual samples, because these composites included material frozen while at an earlier stage of maturity than the average indicates, and such material yielded less flour when milled individually. The low yield of sample C22, exposed to four degrees of frost, can be explained by the fact that it was a single sample cut while containing less than 56% dry matter. It also was grown at the north end of the field, and much of it was greener than is indicated by the average dry matter content. While both the grade and the flour yield of this sample were lower than those of the composite (C23) made up of material exposed to eight degrees of frost, the flour yield was higher than that of any other sample grading "Feed".

The milling results as a whole show that both immaturity and frost exposure caused a reduction in the yield of flour as compared with that obtained from mature, unfrozen checks. The yield of the checks was little affected by the part of the field from which the sample originated, probably because the more immature kernels in the north end samples developed considerably after cutting. The question of such development has been discussed in the second paper (6) of this series. The gradient of reduction in flour yield by increasing severity of frost exposure is, however, steeper in the north end samples. Severe freezing checked translocation almost completely, and kernels which were immature when cut remained so. This reduced the flour yield in such unevenly ripened samples below that obtained from more uniform samples cut at the same average dry matter content. This relation is apparent in the results for both the individual and composite samples.

While both immaturity and frost exposure reduce flour yields, and therefore milling quality, the extent of the effect of frost is determined by the immaturity of the wheat and the severity of the exposure. The flour yield from immature wheat is reduced materially by frost treatment, while the yield even from mature wheat is slightly but definitely reduced.

Baking Quality

INDIVIDUAL SAMPLES

The flour milled from the individual samples of wheat was baked, using the bromate formula (1, 4), one month and again 3½ months after milling. In appraising the quality of the bread produced, records were kept of water absorption, loaf volume, crust color, loaf form, crumb color and texture. The data obtained are too numerous to include in full, but loaf volumes and partial baking scores are presented in Tables III and IV. A full description of the method of scoring the various loaf characteristics is given by Geddes, Malloch and Larmour (4), who also give the method used in computing a single figure baking score. The partial baking score reported in this paper is calculated in the same way except that the score for loaf volume is omitted. The partial baking score is, therefore, a single figure estimate of the loaf characteristics other than loaf volume.

Water absorption is one of the values included in the partial baking score, and the fact that absorption is characteristically high in flour from frosted wheat masks the effect of the frost on the other values included in the score. To offset this, in certain cases indicated in the tables and figures the differences between the absorption of the checks and that of the frosted samples were deducted from the partial baking scores of the latter, in order to put both on a comparable basis with respect to other characteristics.

The results for each character studied, while not presented in detail, have been summarized, and the differences between frozen and unfrozen samples are presented in Fig. 3. The results were divided into three groups: first, those for all samples harvested while containing less than 50% dry matter; second, those for all samples harvested while containing between 50 and 58%; and third, those for all the more mature samples. The results for the samples within each group and each frost treatment were averaged, and these averages used in plotting the curves in Fig. 3.

TABLE III

BAKING RESULTS OF INDIVIDUAL SAMPLES ONE MONTH AFTER MILLING

Dry matter at cutting, %	Loaf volume, cc.			Partial baking score			Partial baking score minus excess absorption	
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost
31.2	450	609	566	26	42	44	24	41
34.9	491	672	564	28	47	41	26	47
39.4	532	627	657	48	53	52	44	49
43.8	427	581	604	45	52	56	37	48
46.8	490	594	656	50	47	55	47	47
48.3*	488	547	590	38	52	53	33	50
50.5*	422	536	564	33	51	54	30	46
51.1*	506	—	593	25	—	55	20	—
51.3*	505	486	553	47	49	56	41	43
55.8*	594	554	598	43	46	55	39	44
56.8	612	672	628	46	57	54	43	55
57.3	622	618	606	56	55	54	54	53
58.1*	656	—	582	60	—	58	55	—
59.1*	644	—	616	58	—	62	55	—
65.5*	618	—	626	58	—	59	58	—
65.6	762	—	611	56	—	59	56	—
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost
34.0	310	336	601	25	22	42	19	18
38.1	322	324	596	22	25	49	12	12
40.9	412	406	622	32	35	55	24	23
45.5	426	448	624	41	48	55	30	36
46.7	537	524	668	46	55	53	37	45
50.5*	454	472	596	36	39	53	29	31
51.1*	460	—	593	27	—	55	24	—
53.3*	598	514	564	28	40	57	25	34
54.9	627	620	652	52	50	57	47	45
57.4*	620	572	535	47	50	57	46	46
58.1*	619	—	582	57	—	58	53	—
58.5	649	644	638	54	56	55	53	55
59.1*	569	—	616	57	—	62	55	—
65.5*	663	—	626	54	—	59	56	—
65.6	742	—	611	53	—	59	54	—
69.4	—	—	672	—	—	62	—	—

**Samples from north end of field.*

The samples falling within the third group were considered as being mature when cut, since in the study of physical (7) and chemical (6) properties, development appeared to cease by the time the dry matter content of the grain had increased to 58%. All other samples were considered as being immature when cut, and were divided into two groups only because the inclusion of the results for the more immature samples with those for the somewhat more mature, would inevitably result in a low average. For convenience they will be referred to as the "immature" and "intermediate" groups, though the latter is definitely immature. As this discussion progresses

TABLE IV

BAKING RESULTS OF INDIVIDUAL SAMPLES 3½ MONTHS AFTER MILLING

Dry matter at cutting, %	Loaf volume, cc.			Partial baking score			Partial baking score minus excess absorption	
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost
31.2	398	531	—	30	43	41	29	43
34.9	380	516	522	39	46	41	38	46
39.4	—	558	586	51	47	49	48	44
43.8	362	540	587	57	51	53	48	46
46.8	419	520	579	53	54	60	49	53
48.3*	470	536	542	45	53	56	40	50
50.5*	452	511	535	42	58	58	38	53
51.1*	396	—	564	44	—	54	35	—
51.3*	—	458	531	55	57	54	48	48
55.8*	525	518	570	39	45	56	37	43
56.8	480	597	600	51	59	54	45	52
57.3	582	659	623	56	54	54	55	52
58.1*	590	—	564	57	—	56	52	—
59.1*	570	—	534	56	—	57	53	—
65.5*	565	—	606	57	—	57	58	—
65.6	646	—	626	55	—	57	55	—
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost
34.0	262	282	450	21	22	36	15	16
38.1	298	324	562	20	24	50	11	12
40.9	338	353	580	28	31	56	21	21
45.5	365	375	568	40	51	60	31	42
46.7	414	347	563	39	44	55	29	33
50.5*	487	405	576	44	46	51	32	33
51.1*	—	—	564	40	—	54	37	—
53.3*	430	452	569	40	47	60	35	42
54.9	544	550	606	50	49	55	43	43
57.4*	564	522	542	33	33	56	31	31
58.1*	562	—	564	54	—	56	50	—
58.5	610	592	638	50	55	52	47	54
59.1*	560	—	534	55	—	57	53	—
65.5*	624	—	606	55	—	57	58	—
65.6	754	—	626	52	—	57	52	—
69.4	—	—	666	—	—	54	—	—

**Samples from north end of field.*

it will be apparent that in many respects the results for the samples in the intermediate group resemble those for the more immature, rather than those for the mature samples.

There were minor differences in the results of the first and second bakings. In general there was a slight decrease in the loaf volume of individual samples, the decrease being greater for immature than for mature, and greater for frozen than for unfrozen samples. There were but small changes in any of the other characteristics scored. The relative values for the various groups and exposures were unchanged as a result of the extra period of storage and, for this reason, the results for the two bakings have been averaged in plotting the curves in Fig. 3.

Loaf Volume

The results for the loaf volumes of the unfrozen checks were in general very uniform, there being comparatively small differences between mature and immature samples. The immature samples decreased somewhat more in loaf volume between the two bakings than did the mature, but the differences were relatively insignificant in most cases.

The loaf volumes of the samples exposed to four degrees of frost were similar to, but on the average slightly lower than those of the checks. The decrease in loaf volume between the two bakings of these lightly frosted, immature samples was only slightly greater than that of their checks. None of them were included in the mature group.

More severe frost exposure caused a marked reduction in the loaf volume of bread produced from immature samples. The volumes of bread from samples in the first group, harvested while containing less than 50% dry matter, were very low and in most cases the bread was of such poor quality as to be unsuitable for human consumption. The effects of 10 and 14 degrees of frost were more marked than those of 8, but 8 was sufficient to cause serious injury. The samples in the intermediate group were not injured as much, and there were no differences in the results for the 8-, 10- and 14-degree exposures (Fig. 3). In general, the effect of frost decreased gradually with increasing maturity of the wheat when frozen. Some of the riper samples of the intermediate group were apparently uninjured by the frost exposure, while the averages for the mature, frozen samples were slightly higher than that for the checks.

The increase in loaf volume due to frost exposure of the mature samples may perhaps be attributed in part to a slackening of the gluten indicated in the gluten scores already reported (6). It is well known that strong flours, fairly high in gluten content as these flours were, require a longer fermentation period than weaker flours for maximum development. It was found that the gluten of flour milled from unfrozen, mature wheat could be slackened considerably by blending the flour with 15% of another flour milled from severely frozen, immature wheat, and that the loaf volume was thereby somewhat increased. This affords one possible explanation of the apparent advantage of the frozen over the unfrozen, mature wheat under the conditions of the test. Probably a more important contributing cause was the greater diastatic activity of the frosted samples (6). Certainly this was the predominant factor in the two samples, cut at 65.6% dry matter, which contributed most to the high average loaf volume of those frozen when mature. These two had a diastatic activity more than 50% greater than their check.

The loaves baked from severely frozen, immature samples were smaller at the time of the second baking than at the time of the first. Deterioration between bakings was more marked in these than in either unfrozen or lightly frozen samples. The mature samples which had been heavily frozen also deteriorated to some extent. The last result, however, was unduly influenced by the values for some of the samples. Others of the severely frozen, mature samples deteriorated little during the three months' storage.

Partial Baking Score

The partial baking score, which is a weighted average of the individual scores summarized in Fig. 3, gives a single figure estimate of all loaf characteristics except volume. Each of the individual characteristics will be briefly discussed before referring to the partial baking score.

The high absorption of the frozen samples has already been noted. Even the samples exposed to four degrees of frost appeared to be affected, and the effect became more marked with an increase in frost exposure. The samples exposed to 10 degrees of frost appeared to be higher in absorption than those

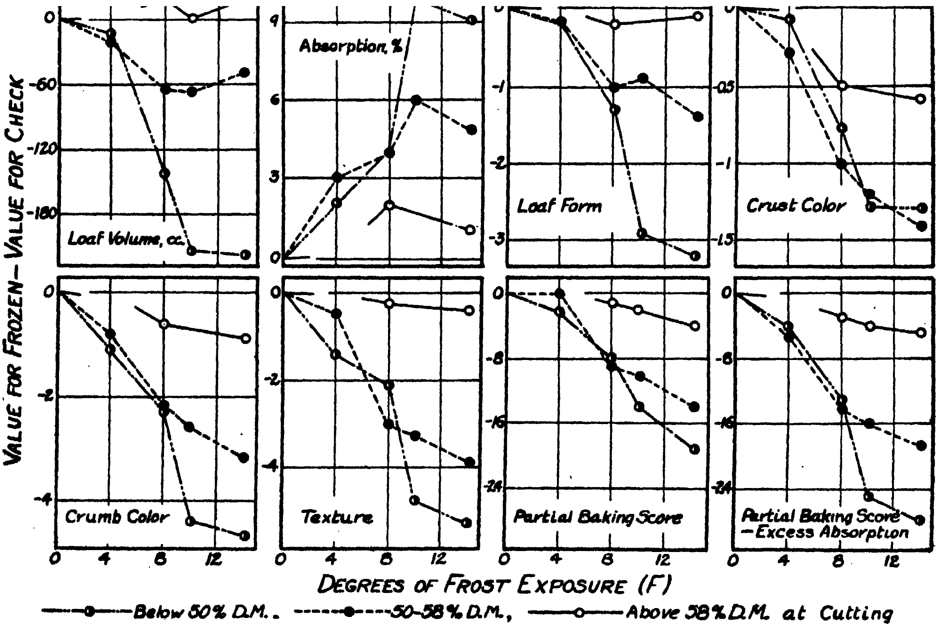

FIG. 3. *Average differences between baking quality of frozen and unfrozen individual samples, within three maturity groups, in relation to frost exposure.*

exposed to 14, but the differences were small and are unaccountable. The absorption of mature frozen samples was only slightly higher than that of the checks.

The results for loaf form show that the more immature samples were distinctly the poorest. The effect of frost increased with the severity of the exposure, but largely disappeared in mature samples.

The crust color of all samples was adversely affected by frost treatment. The immature samples were very high in diastatic activity, and were dark in external color. Many of the severely frozen samples were dull and mottled, the dull appearance being chiefly responsible for the reduction in the color scores of mature samples.

Frost treatment of the wheat affected also the color of the crumb of the bread adversely, the intensity of the effect depending on the maturity of the wheat and the severity of the exposure. The average score for samples of the intermediate group was more nearly like that for the samples of the immature group than like that for mature samples, indicating that the cause of injury to crumb color was operative until the wheat was practically mature.

The texture of the crumb is considered as the most important factor other than loaf volume in determining baking quality. The effect of frost on all immature samples was very pronounced, there being but small differences between the results for the samples in the two immature groups, and some of these differences favored the more immature one. The effect of frost practically disappeared in mature samples, a result in good agreement with those for most other characters studied.

The partial baking scores summarize the five factors discussed above. Except in the two most immature samples the differences between the partial baking scores of the mature and immature checks were very small. The frost affected loaf characteristics, other than volume, very adversely in practically all the immature samples, and the effect on the samples of the more immature group was only slightly greater than the effect on those of the intermediate group. In general, exposure to frost affected the quality of mature samples only slightly.

The defect in the partial baking score as applied to frozen wheat has been pointed out above, namely, that it gives undue importance to the abnormally high absorption of such samples. The curves for the partial baking score minus excess absorption indicate more fully the adverse effect of frost on baking quality than do those for the original score. Since the increased absorption of the frosted samples was proportional to the severity of the exposure, the reduction in their scores by subtracting this was in the same proportion.

The results for mature, frozen samples lend support to the suggestion that the larger loaf volume of a few individual samples must be taken, not as an indication of superiority in these samples, but rather as the result of other factors operating to allow a greater development of the frozen than of the unfrozen samples during fermentation. The lower scores for the frozen samples are an indication of weakness as compared with the unfrozen checks.

The baking results as a whole show that the effect of frost on immature samples was proportional to the immaturity of the wheat and the severity of the frost exposure. Mature samples were but little affected by the frost treatment.

COMPOSITE SAMPLES

The composite samples were baked in three laboratories, using four different formulas. The bromate formula was used in all laboratories and the simple, malt-phosphate and blend-bromate formulas were used one in each laboratory.

TABLE V

BAKING RESULTS OF COMPOSITE SAMPLES ONE MONTH AFTER MILLING

Approximate dry matter at cutting, %	Loaf volume, cc.					Partial baking score				
	Check	4° frost	8° frost	10° frost	14° frost	Check	4° frost	8° frost	10° frost	14° frost
*Bromate formula**										
36	629	667	614	332	325	46	47	44	20	20
45	660	661	528	488	507	53	55	45	41	40
51*	656	613	525	512	559	55	54	43	42	41
57	708	700	674	703	689	53	54	53	52	52
57*	683	613	718	615	693	56	54	51	46	49
66	812	—	843	—	854	54	—	53	—	54
65*	730	—	745	—	795	54	—	55	—	55
Simple formula										
36	530	478	560	418	330	36	33	39	20	18
45	541	457	452	494	620	43	37	44	45	48
51*	532	518	554	522	504	50	48	46	47	41
57	495	518	552	655	699	51	54	51	55	56
57*	509	575	588	639	688	51	52	51	54	54
66	548	—	603	—	694	55	—	56	—	55
65*	536	—	520	—	592	54	—	53	—	60
Malt-phosphate formula										
36	562	612	572	385	385	27	28	29	23	22
45	600	585	510	490	515	32	33	32	33	34
51*	605	598	582	582	510	31	35	32	32	32
57	640	652	660	660	700	41	44	36	41	41
57*	640	635	698	625	705	41	38	31	28	34
66	672	—	700	—	738	40	—	44	—	44
65*	678	—	668	—	738	40	—	42	—	45
Blend-bromate formula										
36	523	570	455	348	328	44	37	40	12	12
45	530	523	470	390	380	57	57	47	34	24
51*	540	500	430	423	415	57	51	36	29	23
57	578	553	538	543	545	51	52	51	54	52
57*	573	512	557	530	558	52	55	52	46	51
66	602	—	592	—	583	54	—	53	—	54
65*	575	—	585	—	570	54	—	55	—	53

**Samples from north end of field.* ***Averages of results from three laboratories.*

The ingredients used in three of these formulas are described in papers published by members of the Associate Committee on Grain Research (1, 4). The blend-bromate formula used was the same as the bromate except that the flour was blended with 50% of a weak, soft-wheat flour. The results with the bromate formula obtained in the three laboratories were averaged, and these averages, together with the results obtained with the other formulas, are presented in Table V. The results obtained for the samples from the south end of the field with the bromate and blend-bromate formas are shown in Fig. 4.

The results obtained by the bromate formula, as might be expected, agree with those for the individual samples, baked by the same formula, and therefore require no discussion. The results obtained when the simple formula was used cannot of themselves be considered a satisfactory indication of the quality of the samples, because this formula does not provide conditions under which the stronger flours may develop to the full extent of which they are capable, while weaker flours may. The principal value of these results lies in the fact that from them the response resulting from the addition of bromate to the formula may be calculated and, since such addition may be considered

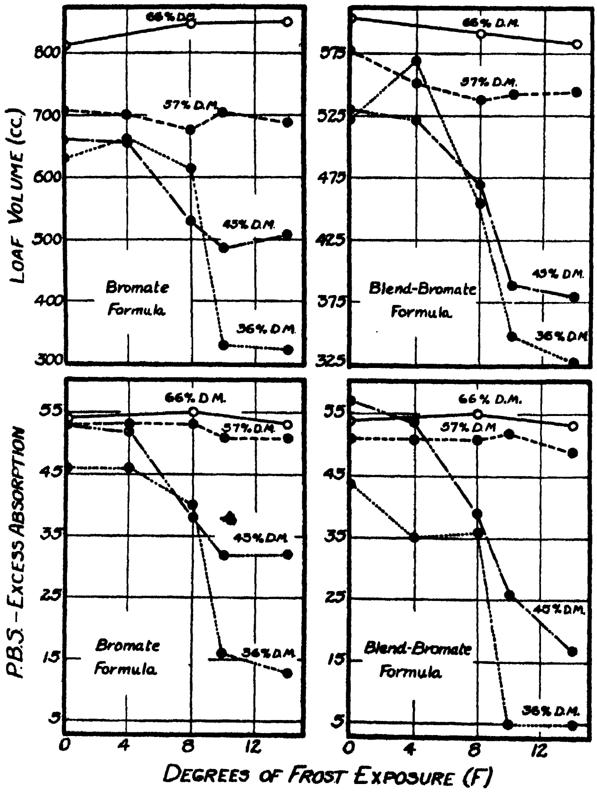

FIG. 4. *Baking quality of "south end" composites, of four stages of maturity, in relation to frost exposure.*

as equivalent to extending the fermentation time, a measure of the reserve strength of the flours obtained. The results for this response are presented in Fig. 5.

The immature check samples gave a greater response to bromate than did the immature samples exposed to 8 degrees of frost, while those exposed to 10 and 14 degrees gave slight negative responses. A discussion of the interpretation of such responses is given by Geddes, Malloch and Larmour (4). Flours giving a strong positive response are regarded as possessing reserve strength, those giving a low response usually show high baking quality by the simple formula and possess little or no reserve strength, and those giving a negative response are considered weak.

On this basis the more immature checks were stronger than the more immature frozen samples, with the possible exception of those exposed to four degrees of frost. The checks harvested while containing 50–58% dry matter were also stronger than the comparable frozen samples. Only in the mature group did the frozen samples exhibit as great reserve strength as the unfrozen checks. It seems possible, however, that other factors such as diastatic activity may have influenced the response to bromate. Nearly all frozen samples were higher in diastatic activity than their checks, and this would tend to increase the bromate response of those mature enough to escape serious frost injury to the gluten.

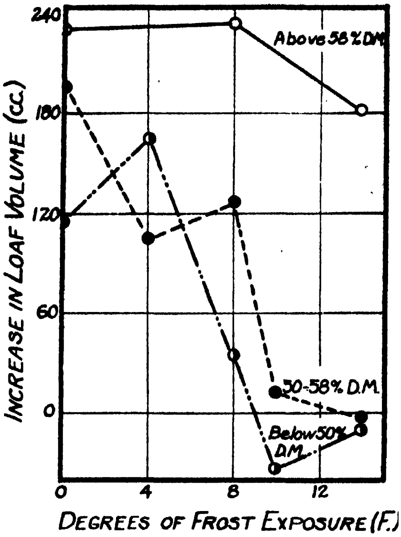

FIG. 5. *Bromate responses of composites, within three maturity groups, in relation to frost exposure.*

The use of malt in the malt-phosphate formula should have had the desirable effect of eliminating diastatic activity as a limiting factor in determining loaf volume. Loaves produced by the check samples using this formula were considerably larger than those obtained with the simple formula but smaller than those obtained when bromate was used. Mature, frozen samples appeared to be better than the mature checks, the addition of malt having caused an increase in the loaf volume of several samples even though the diastatic activity was already high. In experiments done subsequently to those now under discussion, Aitken and Geddes (1) found that either the malt-phosphate or the bromate formula gave increases in loaf volume over those obtained with the simple formula with samples which were fairly low in diastatic activity, but the increase by either formula was less than that when malt and bromate were both used. The interrelation of gas production and gluten development is discussed by these authors, who conclude that the use of an agent such as bromate is necessary to assist in the development of the gluten of only slightly aged, experimentally milled flours, and in addition, conditions must be such that gas production is not limiting. In the present studies, the gas production of the mature checks was limiting when malt was not used, and the development of the gluten was inadequate when bromate was not used. Both of these factors were provided for when the bromate formula was used with the mature, frozen samples, so the results with this formula probably indicate the maximum volume obtainable with these.

The blend-bromate formula yielded results in almost strict accordance with expectation from the knowledge of the frost treatment given the various samples. The soft-wheat flour used in the blends was probably high enough in diastatic activity to insure adequate gas production, and would reduce the optimum fermentation period of the mature samples so that the checks were given the opportunity to develop to their full capacity. These results are in agreement with those for the bromate responses. Frost injury was thus revealed as proportional to severity of exposure and as declining with maturity.

Both loaf volume and partial baking score results show that with all formulas, but especially the bromate and blend-bromate (Fig. 4), the samples harvested while containing an average of less than 50% moisture behaved more like the most immature than like those harvested at an average dry matter content of 57%. There were small but apparently significant differences between the results for samples harvested while containing 57 and 66% dry matter, the latter being superior in all cases. The samples harvested at an average dry matter content of 57% included some material considerably less mature, so the results are not in disagreement with the general conclusion that the baking quality of the wheat was little if any injured by frost exposure after the grain had reached a dry matter content of 58%.

STORAGE PROPERTIES OF FLOUR

The results with the individual samples had suggested that there was a more rapid deterioration of the flour from immature, frozen wheat than of that from the unfrozen checks. Flours from the composite samples milled at the University of Alberta were baked by the bromate formula 1, 7 and 14 months after milling, in the hope that more definite information on this point might be obtained. These samples had been stored at room temperature in tins not tightly sealed. In dealing with the results the samples were again considered in three groups, modified from those used previously (Figs. 3 and 5) in order to bring the north end series C11-15 (Table II) within the more immature group. The groups here are therefore: below 54%, 54–58.2% (to include Sample C25) and above 58.2% dry matter at cutting. The results for each group and each exposure have been averaged and are presented in Table VI. Unfortunately there was insufficient of some of the immature, frozen samples (indicated by asterisks in the table) to complete the third baking. These were the most immature of their respective sub-groups (represented by a single figure in the table), and if their results could have been present to influence the averages, the evidence of deterioration among the frozen, immature samples would undoubtedly have been even more emphatic.

TABLE VI

AVERAGE BAKING RESULTS OF COMPOSITES AFTER STORAGE

Time of storage after milling, months	Loaf volume, cc.					Partial baking score minus excess absorption				
	Check	4° frost	8° frost	10° frost	14° frost	Check	4° frost	8° frost	10° frost	14° frost
Samples under 54% D.M. at time of cutting										
1	619	605	572	495	495	56	56	49	37	36
7	548	544	502	398	407	51	54	45	33	26
14	514	580	409*	310**	285*	50	52	31*	36**	21*
Samples between 54 and 58% D.M. at time of cutting										
1	666	649	710	709	720	55	56	54	51	53
7	566	592	598	580	614	58	57	56	54	54
14	536	557	610	482	562	54	53	52	45	50
Samples above 58% D.M. at time of cutting										
1	758	—	825	—	870	57	—	53	—	53
7	726	—	760	—	698	56	—	55	—	54
14	683	—	674	—	612	58	—	58	—	55

**Only one of three original samples included.*

***Only two of three original samples included.*

There was some decrease with ageing in the loaf volume of samples in each group and at each frost exposure, but the check and four-degree samples of all three groups suffered less than did most of the other samples, and in no instance did the quality fall below the border line of that for acceptable bread. Exposure to 8 degrees of frost resulted in a deterioration of the immature sample, quite marked at 14 months, but the two groups of mature samples showed no greater deterioration than did the checks. Exposure to 10 and 14 degrees of frost induced still more rapid deterioration of the immature samples. There were some indications that the keeping properties of the more mature samples were also deleteriously affected by severe frost treatment. The loaf volumes at the time of the first baking were high, and although the volumes at the time of subsequent bakings were smaller, all of the mature samples produced bread which was acceptable.

In general the results show that the effect of relatively severe frost was so deleterious to the keeping properties of flour milled from immature wheat, that even samples which at first made satisfactory bread became unfit for use after several months' storage. The effect on mature samples was less definite, but the apparent superiority of the frozen samples discussed in earlier sections of this paper had entirely disappeared after the flour had been stored for 14 months.

Discussion

The general effects of maturity and frost on the milling and baking quality of wheat have been discussed in the previous sections. The main purpose of this supplementary discussion is to relate the results reported in this paper to those reported in the earlier papers of the series.

From the producers' point of view, the most important feature of frost damage is the effect on grade. We have shown (7) that even mature wheat was degraded when exposed to frost of varying severity. The decrease in grade was accompanied by a decrease in weight per bushel, but not by a decrease in weight per 1000 kernels. It seems likely that the effect on weight per bushel of mature frozen samples is related to the presence of blisters on the surface of the kernels. It is the presence of these blisters which establishes the fact that the sample has been frozen, and leads to the reduction in grade. The chemical studies (6) showed that the composition of wheat and of flour milled from it was not altered by exposure of the grain to frost after it had reached a dry matter content of 58%. The results of the chemical determinations carried out on the mature samples are not in agreement, therefore, with those for grade, but the stage of maturity represented by an average moisture content of 58% is established as critical in the development of the grain.

The milling results resemble those for grade. The average flour yield obtained from the samples of each grade except "Feed", regardless of the treatment accorded the particular samples included in that grade, are presented in the first section of Fig. 6. The average values plotted in the graph include the yields from both the individual and composite samples, the figure in brackets above each point being the number of individual results included in the average. Only one sample that was exposed to frost graded better than No. 4 Northern, so the results for No. 1, 2 and 3 Northern may be considered as applying only to the unfrozen checks. The yields of flour from

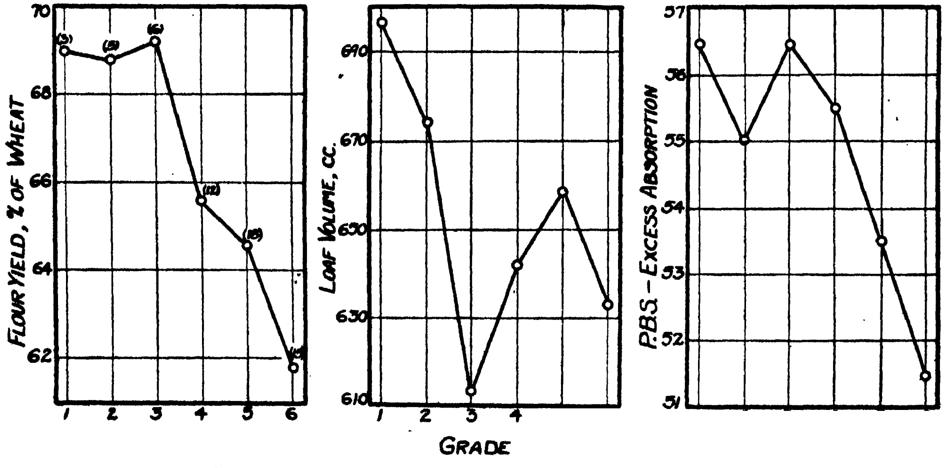

FIG. 6. *Milling and baking quality in relation to grade.*

samples of these three grades were essentially the same, but the effect of frost and immaturity on flour yield was approximately parallel to the effect on grade. These results are in agreement with those obtained by Geddes, Malloch and Larmour (4) with commercial samples.

The relation between grade and baking quality as measured by loaf volume and partial baking score minus excess absorption is shown in the other sections of Fig. 6. The values plotted are averages obtained with the bromate formula for both individual and composite samples. There was greater variation in the loaf volumes of unfrozen checks grading No. 1, 2 and 3 Northern than in those of frozen and immature samples grading No. 4 Northern, No. 5 and No. 6. The average volume of 14 samples grading No. 3 or better was 647 cc., while that of 45 samples grading from 4 to 6 was 646 cc. The comparable averages for partial baking score minus excess absorption were 56.0 and 53.8. These averages are influenced, of course, by the inclusion of results for samples frozen when relatively mature, but 42 of the 59 samples were immature when harvested. While these results indicate that injury to baking quality was not proportional to reduction in grade, one cannot deduce such a conclusion definitely because the baking formula used did not ensure adequate gas production in the higher grades.

These results are not in good agreement with those presented by Geddes, Malloch and Larmour (4), who found that loaf volume in general decreased with grade. Results obtained with other frozen samples suggest that if the protein content of two frozen samples grading No. 6 is, for example, 10 and 15%, the low protein sample will show the effects of the frost very markedly, while the high protein sample may yield good bread. In such cases the higher protein content allows the dough to take fuller advantage of the higher gas production in frozen wheat flour. All of the samples used in the present study were higher in protein content than the average of those studied by Geddes, Malloch and Larmour, and it is possible that this is one reason for the disagreement in results.

The gluten studies (6) indicated that the quality of the protein even of mature samples exposed to frost was slightly reduced. If the relatively mature frozen samples owed their high quality at least in part to the quantity of protein, which afforded sufficient strength to obscure the limited effect of frost on the quality of the protein, the discrepancies between the results for the baking and gluten studies can be reconciled. The indicated deterioration of the heavily frozen, mature samples in storage is of course in line with the observation that the frost had damaged the gluten.

In general, the baking results resemble those obtained in the chemical studies, that is, there was little apparent effect of frost after the dry matter content of the grain exceeded 58%. Although there are indications of slight injury to the baking quality of mature samples, this injury was less than anticipated from the decrease in grade.

The quality results as a whole support the conclusion that the development of wheat is largely completed by the time the dry matter content of the grain reaches 58%. Although frost reduced the grade, weight per bushel and flour yield of more mature samples, the effect of the frost was restricted largely to the outer layers of the kernels.

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GENERAL PRELIMINARY STUDIES ON THE PHYSIOLOGY OF DELAYED GERMINATION IN *AVENA FATUA*[1]

By L. P. V. Johnson[2]

Abstract

A series of general preliminary studies was made on the physiology of delayed germination in *Avena fatua*, the results of which may be summarized as follows: (1) Great variations were found in the after-ripening periods of a number of *A. fatua* selections. (2) Evidence was obtained which strongly indicated that delayed germination is determined by a condition of the seed coat which develops after fertilization. (3) Results from tests of entire panicles indicated a correlation between germinability and the position of the seed in the panicle. (4) The after-ripening period of secondary grains was shown to be much longer than that of primary grains. (5) The placing of incompletely after-ripened grains under germinative conditions induced secondary dormancy. (6) Exposure to light appeared slightly to stimulate germination in seeds which were in the early stages of after-ripening, but appeared to have a harmful effect upon seeds which were more or less completely after-ripened. (7) Low dry-storage temperatures retarded the after-ripening process. Storage in a frozen condition at freezing temperatures resulted in increased germination. Seeds moistened and subjected to outdoor conditions failed to germinate. (8) Dormancy was more or less completely overcome by breaking the seed coat over the embryo, or by soaking seeds in potassium nitrate solutions. The exposure of seeds under germinative conditions to an atmosphere having an increased oxygen concentration definitely stimulated germination. Treatments with pure oxygen, ether, and sodium thiocyanate had more or less indifferent effects upon germination, while ethylene chlorhydrin and dichlorethylene were definitely injurious.

It is inferred from the combined results that delayed germination is due to post-fertilization changes, related either to tissue absorption or development, which occur in the seed coats of *A. fatua* but not in readily germinable species, and which result in a restriction of the oxygen supply to the embryo. It is believed that the after-ripening process may consist, essentially, of a series of changes in the tissues of the seed coat which result in an increased permeability to oxygen.

Introduction

The studies reported herein were undertaken originally with the view of providing a physiological background for an investigation on the inheritance of delayed germination in hybrids of *Avena fatua* L. and *A. sativa* L. Later, however, they were extended to include physiological phases of the problem which had no direct bearing on the genetical work and, as now reported, are considered as general preliminary studies on the physiology of delayed germination in *A. fatua*.

A. fatua, the common wild oat, possesses the property of delayed germination, that is, its seeds will germinate only after a lapse of from several months to a year or more after harvesting. Certain physiological phases of this property were studied by Atwood (1) who reported results which indicated

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that in freshly harvested seeds, oxygen supply was the limiting factor to germination, and that seed coat restriction to oxygen entry probably played a role. The question of the nature of the physiological processes for which oxygen is essential was left open. Garber and Quisenberry (7) also observed that the germination of freshly harvested *A. fatua* seeds was increased by the breaking of the seed coat. Apart from the work of Atwood and of Garber and Quisenberry, no experimental results have been reported, as far as is known, on the physiology of delayed germination in *A. fatua.*

The present work has included the following phases of the problem: comparison of delayed germination in several selections of *A. fatua;* anatomical and physiological bases of delayed germination; relation between position in the panicle, maturity and germinability of seeds; after-ripening periods of primary and secondary grains; development of secondary dormancy; effect of light upon germination; effect of temperature upon germination; and effect of chemical treatments upon germination.

Materials and Methods

Practically all materials trace back to 17 *A. fatua* plants which were selected in 1933 from the experimental fields of the University of Alberta, Edmonton, Alberta, Canada. A few tests were made on the seeds of the original selections, but in most cases material grown separately from each of these plants was used.

The original selections were designated by the letter F followed by the numbers 1 to 17, respectively. Selections from the progeny of the original selections were identified by a number in parentheses. Thus, F-6 (1) represents the first selection from the progeny of original selection number six.

Fourteen of the 17 original selections were typical *A. fatua* plants, two (F-2 and F-15) were selected because of particularly large seeds, while one (F-16) was selected because of a reddish lemma color which differed distinctly from the usual dark brown.

Germination tests, unless otherwise noted in the text, were made in a thermostat-controlled, electrically heated germinator held at 20° C. Only large, mature, sound, primary grains were used, except in the case of special tests of secondary grains. The seeds were tested between the folds of paper pads placed on large blotters which fitted the germinator trays. These pads were easily and cheaply made from white paper towels and proved very satisfactory. Unless otherwise noted, tests were made on 50-seed lots from individual plants, placed in separate pads, and continued for ten days. Where special methods were used, the details are given in the sections of the text devoted to the tests concerned.

Where several test lots were drawn from a single sample, they were counted out by a method designed to eliminate the possibility of selecting the best seeds for a given lot. Such a method was necessary because of the practice of choosing only large, mature, primary grains from the seed stocks of individual plants which were often quickly depleted of the type of seed being selected. The method used may be illustrated by the following example: If four lots of 50 seeds each were to be taken from a single sample, they would be counted out, five seeds at a time, in the following order:

1, 2, 3, 4,
5, 6, 7, 8,
9, 10, etc.

When 40 five-seed groups had been counted out in this manner into four rows of ten groups each, each of the four rows would be bulked separately to give the required four lots of 50 seeds each.

Unless otherwise noted, all material was stored continuously in the laboratory at room temperatures and tested between four and seven months after harvesting.

The reliability of the method of selecting and testing was checked as follows: Ten lots of 50 seeds each were selected from F-6 (bulked) material and tested for germinability (eight months after harvesting) as outlined above. The percentages of germination obtained were, successively, as follows: 24, 20, 16, 18, 14, 12, 14, 16, 20, and 14; the average being 16.8%. These results show that, under the conditions of testing, small differences in the germination of single lots of seeds are not significant. The degree of constancy in this test is, however, believed to be sufficiently good to permit reasonable confidence in the results reported in the present paper.

Experimental Results

Comparison of Delayed Germination in Several Selections

Fifteen selections (F-1 to F-15 inclusive) were tested for germinability in relation to the time elapsing after harvesting. Results from these tests, together with comparisons with the seed weights of the different selections, are given in Table I.

TABLE I

DIFFERENCES IN GERMINABILITY OF *A. fatua* SELECTIONS AT DIFFERENT STAGES OF AFTER-RIPENING, AND COMPARISONS WITH SEED WEIGHT

Material	Weight per 50 seeds, gm.	Germination, %		Material	Weight per 50 seeds, gm.	Germination, %	
		After 5 mo. (approx.)	After 18 mo. (approx.)			After 5 mo. (approx.)	After 18 mo. (approx.)
F-1	1.18	0	46	F-9	0.99	0	6
F-2	1.46	60	100	F-10	1.15	0	50
F-3	1.08	0	74	F-11	1.19	0	92
F-4	1.14	0	12	F-12	1.21	10	80
F-5	1.08	0	0	F-13	1.21	0	45
F-6	1.21	2	78	F-14	1.18	0	80
F-7	1.11	0	50	F-15	1.48	58	100
F-8	1.13	0	54				

These results indicate that there are great differences in the germinability of different selections after five months of after-ripening, that these differences still exist after 18 months of after-ripening, and that there appears to be a positive correlation between weight of seed and germinability at both periods of testing. The correlation between weight of seed and germinability after 18 months was found to be $r = 0.733 \pm 0.081$. Seed weight was based upon 50 primary grains with awns removed. This correlation may be due to natural hybridization between *A. fatua* and the heavier-seeded, readily germinable *A. sativa* forms.

A series of tests was also made at monthly intervals for the purpose of determining the time when germinability commences and the rate of the change from a completely dormant to a completely germinable condition. The results of these tests are given in Table II.

TABLE II

PERCENTAGE GERMINATION IN A SERIES OF TESTS MADE AT MONTHLY INTERVALS

Material	Percentage germination at monthly periods after harvesting						
	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	7 mo.	8 mo.
F-7(1)	0	0	12	12	12	14	24
F-9(1)	0	0	0	2	2	4	—
F-15(1)	0	0	20	28	22	40	40

The results indicate that the period of complete dormancy begins to disappear at about four months after harvest for the more germinable types and later for the less germinable types. After commencing, germinability does not show a steady increase but appears to level off for a few months and then to increase again. Unfortunately, the limited seed supply prevented the extension of the test beyond the eight-month period. Seeds from four *A. fatua* plants selected from the experimental fields at the State College, Pullman, Washington, failed to germinate in any of the several successive tests made between two and eight months after harvesting.

Anatomical and Physiological Bases of Delayed Germination

Atwood (1) concluded that the delay in germination of *A. fatua* seeds was occasioned by a restricted oxygen supply to the embryo due to the obstruction of oxygen entry by the "seed coat." This conclusion infers that the direct cause of delayed germination lies in a condition of non-embryonic tissue.

In the present work, the question of whether the direct cause of delayed germination was embryonic or non-embryonic in nature was attacked from three angles: (*i*) by germination tests of hybrid (or F_0) seeds arising directly from crosses between *A. fatua* and *A. sativa* in which *A. fatua* was the maternal parent; (*ii*) by observations on the germinability of seeds from hybrid plants; and (*iii*), by experiments in which *A. fatua* seeds were dehulled and variously treated with respect to the "seed coat" (pericarp and testa).

(*i*) Several crosses were made between a number of *A. fatua* lines [F-6(2), F-9(2), and F-15(1)] and *A. sativa* var. Victory in which the former were the maternal parents. Twelve hybrid seeds were produced in this manner. (The variety Victory is capable of complete germinability soon after harvest). As germinability is the dominant character in such crosses (7), the effect of crossing is actually the placing of germinable embryos within the extra-embryonic parts of *A. fatua* seeds. In all cases, seeds arising directly from crossing germinated when tested 75 days after harvesting, while *A. fatua* parental checks did not germinate. The germinability of the hybrid seeds cannot have been due to the effect of tissues developed prior to or independently of fertilization, since such tissues would be the same in the *A. fatua* parent. It must be concluded, therefore, that germinability was brought about through the influence of the *sativa* elements in the zygote, the inference from which is that any tissues concerned must have been developed after, or influenced by, fertilization.

Experiments were conducted to determine the effect of emasculation and pollination upon the germinability of *A. fatua* seeds. Florets were opened as in emasculation, one anther removed from each and the panicles covered, as in the actual hybridization work, with a glassine bag. Two or three days later the florets were again opened, simulating the mechanical part of pollination, and the bags replaced. This procedure was found not to have stimulated germinability when germination tests were made subsequently upon 50 seeds.

(*ii*) Further support for the above conclusions is afforded by the fact that segregation for germinability was observed among the seeds of single plants in the F_1 and other generations from crosses between *A. fatua* and *A. sativa*. The only possible explanation of such segregation lies in the assumption that the recombination of genetic factors brought about by the self fertilization of each floret had an immediate influence upon germinability—which can only mean that germinability is determined by tissues or other causal agencies developed after, or influenced by, fertilization.

(*iii*) Results from the third experiment provide additional information regarding the factors determining germinability.

Seeds from a number of selections were dehulled with great care by a process of peeling the hulls from the caryopses. Where the dehulled seeds were to receive no further treatment, they were dropped gently from the hulls directly to the germinator pad. The dehulled seeds were given three different treatments: one group of seeds was rolled with considerable force between the thumb and forefinger, another was scraped over the embryo with a razor blade, and a third group received no treatment except very careful dehulling. The results of germination tests of the variously treated seeds are summarized in Table III.

The tests of F-1, F-2, F-3, F-4 and F-5 were made in 1933, 20 seeds being used for each test. In 1934 an attempt was made to repeat the experiment on a larger scale; but, because of high germinability of the remainder of the material tested, only F-13(3) gave significant results. The results from F-13(3) are included with the previous data in Table III.

TABLE III

PERCENTAGE GERMINATION OF A NUMBER OF *A. fatua* SELECTIONS AFTER VARIOUS TREATMENTS OF THE HULL AND SEED COAT

Material	No. of seeds per test	Treatment			
		Hulled	Dehulled	Rolled	Scraped
F-1	20	0	0	45	—
F-2	20	60	60	100	—
F-3	20	0	0	—	100
F-4	20	0	0	—	100
F-5	20	0	0	85	—
F-13(3)	50	0	6	30	56

It may be concluded from the data in Table III that very careful dehulling (removal of lemma and palea from the caryopsis) has no appreciable effect upon germinability; but that general injury to the cuticle, pericarp and testa (as by rolling), or definite breaking of these structures over the embryo, results in a marked stimulation of germination. These results, considered in the light of previous conclusions, would indicate that delayed germination is brought about by the tissues covering the embryo, the character or development of which is influenced by the genetic recombinations resulting from the fertilization of the floret in question.

It is known that after fertilization great modifications take place in the tissues enveloping the embryo. Robbins (13) states that before fertilization the enveloping tissues of the oat seed consist of the following, named successively from the outer to the inner: outer epidermis, parenchyma layer, chlorophyll layer, inner epidermis, outer integument, inner integument, and the nucellus. After fertilization, all of these tissues are more or less completely absorbed, with the exception of the outer epidermis and the inner integument. In the mature seed, the outer epidermis with remnants of the parenchymatous layer forms the pericarp, while the inner integument becomes the testa. The pericarp and testa are fused to form the "seed coat".

It is concluded on the basis of the above results and discussion that delayed germination in *A. fatua* is due to agencies, operating after and influenced by fertilization, which affect the development or absorption of tissues enveloping the embryo in such a manner as to prevent germination of the seed until after a certain period of after-ripening has elapsed.

Relation between Position in the Panicle, Maturity and Germinability

The possibility of germinability being affected by the position of the spikelets in the panicle was investigated, together with observations on the effect of seed maturity.

The F-12 line was selected for study because of its intermediate degree of germinability (see Table I.). It was attempted to procure a panicle in which all spikelets were completely ripened and still retained in position, by means of placing the panicle between screens which prevented the dropping of mature grains. Unfortunately, when the panicle so treated was tested only one seed germinated.

Several other panicles at various stages of maturity were harvested from the progeny of Selection F-12, the most mature being at the stage when the upper seeds had begun to drop. Germination tests were made five and one-half months after harvesting.

In a general way, all panicles showed the same manner of germination with respect to panicle position, regardless of the stage of maturity; that is, seeds in "germinable positions" in immature panicles germinated even though less mature than seeds in "non-germinable positions" in mature panicles. Table IV serves to indicate the relation between the point of occurrence in the panicle and the germinability of the seed in a typical panicle of the most mature type. The "shelled out" seeds mentioned in the table were those dropped from the panicle in handling, and were for the most part from the tips of the longer branches of the upper whorls.

TABLE IV

RELATION OF POSITION IN THE PANICLE TO GERMINATION OF PRIMARY AND SECONDARY FLORETS OF F-12(2)

	Lower whorl		Second whorl		Third whorl		Upper whorls		Seeds shelled out	
	Pri.	Sec.	Pri.	Sec.	Pri.	Sec.	Pri.	Sec.	Pri.	Sec.
No. of seeds	13	12	13	10	15	10	19	8	7	2
No. germinated	2	0	3	0	9	0	13	0	6	0
Germination, %	15.4	0.0	23.1	0.0	60.0	0.0	68.4	0.0	85.7	0

It will be noted that there was a progressive increase in the germinability of primary grains in passing from the lower to the upper, earlier-maturing whorls. Where seeds from lower whorls germinated, they were in all cases from the tips of the longer branches, the points of earliest maturity for the whorls in question. Thus, in a given panicle, position and maturity were correlated; and both, in turn, were correlated with germinability. The germination of relatively immature seeds in the upper whorls and branch tips in the two immature panicles tested would indicate that position and not maturity was the factor primarily correlated with germinability.

It will be observed that in no case did secondary grains germinate.

In *A. sativa* var. Victory it was found that very immature seeds, both primary and secondary, germinated almost as well as fully matured seeds, regardless of position in the panicle.

After-ripening Periods of Primary and Secondary Grains

In the study of germinability in relation to the position of seeds in the panicle, it was found that secondary grains did not germinate, a fact which led to an investigation of possible differences in the length of the after-ripening periods of primary and secondary grains.

After individual-plant material was selected, all spikelets with both primary and secondary seeds intact were examined. Those with both grains sound and reasonably mature were selected and the primary and secondary grains separated for testing. Fifty primary and 50 secondary seeds were also selected from the threshed-out seed of each plant. The results from tests made seven months after harvesting are presented in Table V. Relatively lower germination was obtained from seeds of the selected spikelets, since the spikelets retaining both grains intact tended to be less mature and from lower positions in the panicle than the threshed-out seeds.

TABLE V

DIFFERENCE IN GERMINATION OF PRIMARY AND SECONDARY GRAINS SEVEN MONTHS AFTER HARVESTING

Material	Seeds from same plant			Seeds from same spikelet		
	No. of seeds each sample	Germination, %		No. of seeds each sample	Germination, %	
		Pri.	Sec.		Pri.	Sec.
F-6(4)	50	0	0	33	0	0
F-12(2)	50	16	0	25	12	0
F-15(1)	50	48	0	20	30	0
F-15(6)	50	58	0	20	30	0
Ave. germination, %	—	30.5	0.0	—	18.0	0.0

These data show unmistakably that there are very marked differences in the germinability of primary and secondary grains. However, such differences might be due to smaller size or other factors which may tend to lower the vitality of secondary grains. If the lack of germination were due to true

TABLE VI

DIFFERENCE IN GERMINATION OF PRIMARY AND SECONDARY GRAINS 18 MONTHS AFTER HARVESTING

Material	Seeds from same plant			Seeds from same spikelet		
	No. of seeds each sample	Germination, %		No. of seeds each sample	Germination, %	
		Pri.	Sec.		Pri.	Sec.
F-2	50	100	84	15	100	93.3
F-6	50	78	20	—	—	—
F-10	50	50	0	—	—	—
F-12	50	80	40	10	100	30
F-15	50	100	82	10	100	100
Ave. germination, %	—	81.6	45.2	—	100	74.4

dormancy rather than to low vitality, it would be expected that after a more extended period of after-ripening, the secondary grains would show a capacity to germinate. The test was, therefore, repeated using older material, including seeds from the progenitorial plants of the material used in the first test. The results from the second test in which the material used was 18 months old are given in Table VI.

The second test shows that secondary grains are capable of high germinability when given a sufficient period of after-ripening.

It is concluded from the results of the two tests that secondary grains of *A. fatua* require a much longer period of after-ripening than primary grains. The survival value in nature of such a difference would lie in the fact that the seed from a given plant would provide propagules for at least two successive seasons.

A similar adaptation is found in *Xanthium*, the cocklebur, where the so-called lower seed germinates the first spring after maturity while the upper seed remains until the following spring or even later before germinating (4).

Development of Secondary Dormancy

The almost complete lack of germination in re-tests of dried, non-germinated seeds from previous tests made it quite obvious that the placing of insufficiently after-ripened seeds under germinative conditions caused the development of a form of secondary dormancy. Secondary dormancy has been observed in the seeds of a number of other species (3, 10). The term is used to distinguish between an induced dormancy and the primary dormancy present at maturity in the seeds of certain plants.

In order to study further the matter of secondary dormancy in *A. fatua*, seeds were retained from tests made about four months after harvest, stored in a dry condition for three months, and then again tested, together with continuously dry-stored seeds from the same respective seed stocks. The results of these tests are given in Table VII.

TABLE VII

DATA INDICATING THE DEVELOPMENT OF SECONDARY DORMANCY THROUGH PLACING INSUFFICIENTLY AFTER-RIPENED SEEDS UNDER GERMINATIVE CONDITIONS

Material	Tested 4 months after harvesting		Non-germinated seeds from previous test, re-tested 3 months later		Continuously stored seeds, tested 7 months after harvesting	
	No. of seeds	Germination, %	No. of seeds	Germination, %	No. of seeds	Germination, %
F-6 (bulked)	50	0	25	0	25	16
F-8(3)	50	0	50	0	50	22
F-9(1)	50	0	50	0	50	4
F-12(2)	50	0	50	0	25	16
F-13 (bulked)	50	0	25	0	25	4

The difference in the treatment of the re-tested seeds and continuously stored seeds is that the former received, in the form of a germination test, a ten-day break in the continuity of dry-storage conditions. Such a short period of time removed from the after-ripening period cannot account for the observed reductions in the germination of the re-tested seeds. It was shown by special tests that seeds which were non-germinable in early tests had not suffered appreciable loss of vitality but were highly germinable (up to 76 per cent) when given an after-ripening period sufficient to overcome the state of secondary dormancy. It must be assumed, therefore, that the non-germinability of the re-tested seeds is due to a form of secondary dormancy induced by the conditions imposed by the first germination test.

Effect of Light upon Germination

The effect of light on the germination of seeds has been studied by a number of investigators. Gardner (8) found that exposure to light caused a stimulation of germination in the seeds of *Rumex crispus* and *Phoradendron flavescens* and a retardation of germination in the seeds of *Datura stramonium*. It has been reported by Morinaga (11) that light and nitrogen compounds have a favorable joint effect on the seed germination of Bermuda grass (*Cynadon dactylon*), Canada blue grass (*Poa compressa*), and cat-tail (*Typha latifolia*). Johnson *et al.* (9) found that light was necessary for the germination of some types of tobacco (*Nicotiana*) seed, but that the seeds of most of the common American varieties would germinate in the dark, although in some cases at a slower rate and with a lower percentage than when light was present.

The studies on the effect of light upon the germination of seeds of *A. fatua* were on the whole very inconclusive and, in consequence, the results are not tabulated. Both hulled and dehulled seeds were used in all tests.

A test was made using both glass and common dark germinators. During the day the glass germinator was in diffused daylight while during the night it was in practically complete darkness. In each of the four seed stocks used, the seed tested in the light gave considerably (about 15%, on the average) higher germination in the case of both hulled and dehulled seeds.

The temperature in the dark germinator during the test was approximately 20° C. while that in the glass germinator was one degree higher. Aeration may have been slightly better and humidity slightly lower in the glass germinator. It is questionable whether these differences could account for the observed differences in germination results. However, the fact that seeds with hulls intact showed increased germination in light would lead one to suspect that factors other than light were operative.

Further tests were made to determine the effects of alternate and continuous diffused light and continuous direct light upon germination. The source of continuous light was a common desk lamp with a 50-watt bulb. Direct light was obtained by placing the glass cylinder containing the seeds about 30 inches from the lamp and directly in front of it; diffused light was obtained by placing a similar cylinder the same distance to the side of the lamp out of the line of direct illumination. The glass cylinders used in these tests were twelve inches in height and seven inches in diameter, and were well-aerated and maintained at a favorable humidity by a free-water surface. Alternate diffused light was obtained in the glass germinator as described in the previous experiment. The approximate temperatures maintained in the various tests are as follows: dark, 20° C.; alternate diffused light and continuous diffused light, 21° C.; continuous direct light, 21.5° C.

The results from this test, in contradiction to those of the preceding test, showed that in general all forms of light were detrimental, particularly continuous direct light. The fact that the seeds used in the first test were in the early stages of after-ripening while the seeds of the second test were in an advanced stage of the process suggests that light may have a stimulating effect under the former conditions and a detrimental effect under the latter conditions. Unfortunately, a lack of suitable materials prevented repetition of the experiment.

The Effect of Temperature upon Germination

A study was made of the effect of prolonged storage of wet and dry seeds at different temperatures upon subsequent germination.

After storage in the laboratory for four months, four lots of 50 seeds each were drawn from each of three different seed stocks. One lot from each seed stock was then placed under each of the following conditions: (1) continued dry storage in the laboratory; (2) moistened and maintained in a frozen state in a refrigerator held at slightly below 0° C.; (3) dry storage in an unheated seed-house vault where the temperature probably varied from a few degrees above freezing to about −18° C.; and (4) moistened and stored in moist sand in the open, shaded from the sun during the middle of the day, and experiencing temperature variations between 11° and −25° C. Storage under these various conditions extended from Dec. 6, 1934 to March 6, 1935—a 90-day period. The results of the germination tests which were begun on March 6 are summarized in Table VIII.

TABLE VIII

COMPARISON OF GERMINATION OF SEEDS STORED FOR 90 DAYS UNDER DIFFERENT CONDITIONS, NAMELY, IN HEATED LABORATORY, IN REFRIGERATOR AT 0° C. (FROZEN), IN UNHEATED SEED HOUSE, AND IN THE OPEN

Material	Percentage germination following various kinds of storage			
	Laboratory	Refrigerator	Seed house	In the open
F-12(41)	12	14	2	0
F-15(2)	18	16	4	0
F-15(5)	32	74	12	0
Average	20.7	37.7	6.0	0.0

The results indicate that the after-ripening process in dry seeds was much slower at temperatures around the freezing point (seed-house conditions) than it was at about 21° C. (laboratory conditions). Unfortunately, germination tests were not made at the time of subjecting the seeds to the different storage conditions. Such tests would have indicated the stage of after-ripening at the beginning of the storage period, and thus would have made it possible to determine the extent to which after-ripening occurred at the different temperatures. It is possible that the after-ripening process was barely, if at all, active at the seed-house temperatures.

In the case of storage in the frozen condition (refrigerator), it does not seem reasonable to assume that the rate of after-ripening was greater than at laboratory temperatures; but, rather, it would seem quite reasonable to assume a more or less complete cessation of the after-ripening process in the frozen seeds. Therefore, an increase in germination due to storage in the frozen condition is probably best explained by assuming a relatively sudden breaking of dormancy occasioned by the effects of freezing or of thawing or of both.

The fact that the seeds stored in the open did not germinate is perhaps the result of the development of secondary dormancy through the occurrence of germinative conditions for short periods of time, such as warm days, followed by conditions, such as cold nights, which definitely checked any tendencies toward germination. (See section on secondary dormancy.) Freezing and thawing have, apparently, no permanently injurious effects upon germinability, for seeds of *A. fatua* regularly undergo these conditions in the natural environment of temperate regions.

Effect of Chemical Treatments upon Germination

Investigations were made in which the effects of the following chemicals upon the germinability of dormant seeds were tested: oxygen, ether, potassium nitrate, ethylene chlorhydrin, dichlorethylene, and sodium thiocyanate.

The effect of oxygen concentration upon germinability was tested in airtight chambers formed by inverting two-litre Petri dish covers on special supports in a large copper pan partly filled with water. The supports were constructed in such a manner as to hold the seed containers (Syracuse watch glasses) above the water and to permit necessary manipulatory operations.

In the case of the pure oxygen test, the procedure was as follows: Syracuse watch glasses containing the seeds were filled with water to exclude the air, and each covered by a second watch glass and placed under the Petri cover. The air was then sucked from the chamber by means of a U-shaped tube until the chamber was completely filled with water. Oxygen was generated by heating a mixture of potassium chlorate and manganese dioxide, and passed into the chamber until the water was displaced to a level well below the seed containers. The watch glass covers were removed from the seed containers by wire manipulators, and the excess water sucked from the containers by a specially constructed tube.

The procedure in the case of a second set-up was essentially the same, except that only half of the air was removed from the chamber. Thus, the atmosphere in this chamber consisted of air and pure oxygen in the ratio of 1 : 1.

A third and similar set-up was used to test the effect of an atmosphere receiving one drop of ether daily, while a fourth served as an air check.

All chambers were supplied with a free liquid surface of sodium hydroxide as a precaution against the accumulation of an excess of carbon dioxide from the germinating seeds. The oxygen concentration chambers were not disturbed at any time during the test. Watering was accomplished by means of a specially designed glass tube, while germinated seeds were removed by wire manipulators. The ether and check chambers were removed and replaced daily.

The results are summarized in Table IX. Each lot consisted of 50 seeds.

TABLE IX

GERMINATION IN DIFFERENT ATMOSPHERES CONTAINED IN AIR-TIGHT GLASS CHAMBERS

Atmosphere	Germ. of F-15 (bulked), %			Germ. of F-18 (bulked), %		
	Lot 1	Lot 2	Average	Lot 1	Lot 2	Average
Air	36	32	34	4	6	5
Air : Oxygen = 1 : 1	40	38	39	26	12	19
Oxygen	32	40	36	6	2	4
Air-ether	48	52	50	0	0	0

From these data it would appear that an atmosphere of pure oxygen had little or no stimulatory effect upon germination, and that the 1 : 1 air-oxygen mixture gave slight stimulation to the F-15 seeds and considerable stimulation to the F-18 seeds. Ether, as applied in the test, appears to have stimulated germination in the F-15 seeds and to have inhibited it in the F-18 seeds.

The results from the 1 : 1 air-oxygen test are in agreement with those of Atwood (1) who obtained increased germination by subjecting *A. fatua* seeds to increased oxygen concentrations.

As a result of Morinaga's (11) report of the stimulatory effect of solutions of potassium nitrate upon the germination of the seeds of certain grasses, this chemical was tested for its effect upon *A. fatua* seeds. As a preliminary test, seeds of F-15 (bulked) were soaked for 48 hr. in potassium nitrate solutions of different concentrations as well as in tap water. The germination percentages after soaking in 0.2, 0.5, and 1.0% solutions were 70, 92, and 96, respectively, while 10% germination occurred in the water check.

The results show a very marked stimulation as a result of soaking in potassium nitrate solutions, with a consistent increase in germination as the concentration was increased from 0.2 to 1.0%.

TABLE X

PERCENTAGE GERMINATION AFTER SOAKING SEEDS IN WATER, AND IN 10 CC. OF KNO_3 OF DIFFERENT CONCENTRATIONS FOR 24- AND 48-HOUR PERIODS

Material	Water		0.5% KNO_3		1.0% KNO_3		2.0% KNO_3	
	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.
F-6 (bulked)	0	0	2	2	20	6	64	22
F-15 (bulked)	4	0	18	8	74	18	68	56

These preliminary results suggested a further test in which the concentration of the solution was increased and the time of soaking varied. The data from such a test are given in Table X.

In all cases soaking for 24 hr. gave higher percentages of germination than soaking for 48 hr. In the case of F-6 seeds the 2% concentration gave the highest germination, while in the case of F-15 the 1% concentration was the most stimulatory.

A test was next made to determine the effect of soaking for shorter periods of time in 1% and 2% solutions, the results of which are given in Table XI.

It may be concluded from data given in Table XI that the process of stimulation, which is presumably related to the absorption of K^+ or NO_3^- ions by the embryo, required twelve hours to reach maximum effectiveness in the case of .1%, and six hours in the case of 2% solutions. It would appear that periods of soaking up to six hours in the 1% solution had no effect upon germination, while a one-hour soaking in the 2% solution produced a marked stimulation.

TABLE XI

EFFECT OF TIME OF SOAKING IN 25 CC. OF 1% AND 2% SOLUTIONS OF KNO_3 UPON GERMINATION OF F-15 (BULKED)[1]

Solution	Number of hours of soaking				
	1	3	6	12	24
Water	74	—	—	—	—
1% KNO_3	78	70	72	100	98
2% KNO_3	96	96	100	100	100

[1] *The F-15 material used in this test represents a different group of bulked plants and is several weeks older than the F-15 material used in the test reported in Table X.*

A test was next devised to determine the effect of soaking seeds for a constant length of time in different volumes of a relatively dilute solution of potassium nitrate, the results of which are summarized in Table XII.

From these data it would appear that the stimulatory effect upon germination was increased by increasing the volume of the relatively weak solution of potassium nitrate in which the seeds were soaked. Further, this increase in stimulation appears to be similar to that obtained when the concentration of a constant volume is increased (See Table X), and to that obtained when the period of soaking is increased in a constant volume of constant concentration (See Table XI). It may be deduced from these observations that the stimulation of germinability is due to the absorption of either the K^+ or NO_3^- ion by the embryo or related tissues, the greater the number of ions absorbed up to a certain point the greater being the stimulation. The

TABLE XII

EFFECT ON PERCENTAGE GERMINATION OF SOAKING SEEDS OF F-15 (BULKED) 48 HR. IN DIFFERENT VOLUMES OF .125% SOLUTION OF KNO_3

Water	0.125% KNO_3				
	10 cc.	20 cc.	40 cc.	80 cc.	160 cc.
74	78	80	100	94	100

absolute amount of ions absorbed, until the maximum is reached, is determined by the total number of ions present in the solution whether concentrated in a small volume or dispersed in a large volume, and by the amount of time which is allowed for absorption to take place.

The probability that the NO_3^- rather than the K^+ ion provides the stimulation is indicated by the fact that Morinaga (11) found that, in addition to potassium nitrate, several other nitrate compounds produced increased seed germination.

A number of other chemicals, namely, ethylene chlorhydrin ($ClCH_2CH_2OH$), dichlorethylene ($C_2H_4Cl_2$), and sodium thiocyanate (NaCNS), were tested for effect upon the germination of seeds of *A. fatua*. Denny (5) has reported each of these chemicals to be effective in forcing the sprouting of dormant potato tubers. The same author found (6) that ethylene chlorhydrin vapor also broke the rest period of freshly harvested gladiola corms. Ethylene chlorhydrin was found by Deuber (2) to be effective also in shortening the rest period of sugar maple, Norway maple, black oak, and red oak seeds.

In the present experiments, the following methods of seed treatment were used for the different chemicals:

In the cases of ethylene chlorhydrin and dichlorethylene, the seeds were immersed in 100 cc. of the solution for one minute, after which the solution was drained off and the bottle stoppered for 24 hr. When sodium thiocyanate was used, the seeds were soaked for one hour in 100 cc. of the solution, drained and placed directly upon germinator pads. The concentrations of solutions and duration of treatment used were based upon the type of treatment found most effective by previous investigators. In all cases the germinator pads were moistened thoroughly with water before receiving the seeds, and subsequently kept moist with water.

The results of these tests are given in Table XIII.

TABLE XIII

EFFECT OF VARIOUS CHEMICAL TREATMENTS UPON THE PERCENTAGE GERMINATION OF F-12 (BULKED)

H_2O, 1 hr.	$ClCH_2CH_2OH$			$C_2H_4Cl_2$				NaCNS			1% KNO_3, 24 hr.
	1%	3%	6%	0.1%	0.2%	0.5%	1.0%	1%	2%	3%	
48	0	4	0	28	24	36	0	56	50	32	96

The results of the chemical treatments, considered in relation to the results of the water check, indicate: (*i*) complete or nearly complete inhibition of germination in the case of ethylene chlorhydrin in all concentrations used; (*ii*) a complete inhibition by the 1% dichlorethylene treatment and a moderate inhibition by treatments with weaker concentrations; and (*iii*), a slight stimulation by the 1% sodium thiocyanate treatment, with the 2% treatment indifferent and the 3% treatment moderately inhibitory. A potassium nitrate check gave almost perfect germination.

The results from the sodium thiocyanate treatments indicate the possibility of stimulation at concentrations below 1%.

General Discussion and Conclusions

The experimental results have been, with respect to the more specific points, more or less fully discussed in the sections devoted to the different experiments.

In considering at this point the implications and relations of the experimental results, it is recognized that the general and preliminary nature of the work does not permit any extensive generalization. There are, however, some points which should be discussed from the point of view of the work as a whole, particularly those concerning the causes of delayed germination and the stimulation of germination in dormant seeds.

It was shown by studies on the germinability of hybrid seeds that delayed germination is, in all probability, due to agencies operating after, and influenced by, fertilization. The results from experiments on the effect of breaking the seed coat over the embryo indicated very strongly that the seed coat (pericarp and testa) was intimately concerned with the delay in germinability. Considering these two facts together, it would appear that certain agencies affecting the seed coat during the development of the seed have a causal relation to delayed germination.

Post-fertilization agencies which occasion the absorption of several distinct tissues which envelop the ovule before fertilization are known to be operative in the oat seed (13). It may be inferred, therefore, that the property of delayed germination in *A. fatua* may be due to an inherent tendency to undergo a different type or a lesser degree of post-fertilization absorption of germination-restricting tissues enveloping the embryo than occurs in readily germinable species. A less probable inference is that delayed germination is due to an inherent capacity of post-fertilization development of tissues capable of preventing immediate germinability.

The question now arises: how do these tissues bring about delayed germination and what effect has the after-ripening process upon them? The results of the present work agree with those of Atwood (1) in indicating that an increased oxygen concentration in the atmosphere stimulates germination. Atwood also found that when seeds were subjected to increased oxygen concentrations, or when seed coats were broken, there was an absolute increase in the rate of oxygen absorption. It is universally recognized that a supply of oxygen is a prerequisite to seed germination. There appears, therefore, to be a strong probability that the tissues in question cause delayed germination by restricting the oxygen supply to the embryo. Thus, the after-ripening process may be considered as being essentially a series of changes in the tissues enveloping the embryo which results in an increased permeability to oxygen.

The observation that germinability may be correlated with the position of the seed in the panicle, could be explained by assuming that in the case of seeds in "germinable positions", which are also positions of early maturity, the process of post-fertilization absorption of tissue is carried further than it is in the case of seeds in other positions in the panicle. This line of reasoning may also be applied in an explanation of the germinative differences between primary and secondary grains.

The whole question of post-fertilization tissue absorption or development is in a hypothetical condition; it suggests certain procedures for further investigation, especially along anatomical and microchemical lines.

Of the seed treatments used in the present work, the most efficient in overcoming dormancy were: (*i*) breaking the seed coat over the embryo, (*ii*) soaking the seeds for 12 to 24 hr. in 1% or 2% potassium nitrate solutions and (*iii*) subjecting seeds under germinating conditions to an atmosphere having an increased concentration of oxygen. The reasons for stimulation of germination in the case of coat-breaking and oxygen-concentration treatments are obvious from the above discussion. The stimulation by potassium nitrate might be considered as resulting from: (*a*) direct germinative stimulation by the absorbed anion; (*b*) indirect effects of the absorbed anion such as, for example, increased utilization of stored carbohydrates (12), or, the acceleration of organic acid formation following reduction; and (*c*) chemical effects upon the seed coat which produce increased permeability to oxygen.

Freezing of the seed also produced a certain degree of stimulation, which is to be expected on the basis of an increased permeability to oxygen through ruptures in the seed coat resulting from frost injury.

The development of secondary dormancy in partly after-ripened seeds may represent the re-establishment of a high degree of impermeability in seed coats which had nearly reached a state of permeability to oxygen.

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ASSOCIATIONS OF SOMATIC CHROMOSOMES INDUCED BY HEAT AND CHLORAL HYDRATE TREATMENTS[1]

By F. H. Peto[2]

Abstract

Germination of *Hordeum vulgare* at 35–36° C. induced, in the nuclei of the root tips, numerous fractures in one or both chromatids, particularly in the attachment region of the chromosome. The fractured ends appeared to possess an unsatisfied attraction for each other. Chiasmata were formed by the union at random of the fractured ends which happened to lie close together in the nucleus, giving rise to associations of one and one-half, two, and three chromosomes involving chromatid interchange or crossing-over between non-homologous as well as homologous chromosomes. The manner in which fragmentation, translocation, and elimination of chromosome parts can occur in somatic tissue is demonstrated. Somatic segregation and the incidence of new linkage relationships are discussed.

The formation of tetraploid and octaploid nuclei was induced in root tips of *Pisum sativum* by treatment with chloral hydrate. The chromosomes of these polyploid nuclei were frequently closely associated in pairs which bore a superficial resemblance to the paired associations observed in barley. The chromosomes, however, were never united by chiasmata and retained their juxtaposition solely through their inability to separate normally at anaphase.

Introduction

Pairing of chromosomes in somatic divisions occurs in a large number of plant and animal species. Watkins (19) lists 32 species of plants in which this phenomenon is known to occur and Metz (13, 14) reports somatic pairing of homologues in approximately 80 species of *Diptera*. In the majority of cases this pairing is simply a side-by-side association of homologues or daughter chromosomes without any evidence of their being united by chiasmata. In many instances this juxtaposition is maintained by virtue of homologous attraction as is the case in *Diptera*. In others the paired relationship is initiated by faulty separation of the daughter chromosomes after equational division.

In meiosis of most plants and animals the paired condition of the homologues is maintained between diplotene and the beginning of anaphase by means of chiasmata. A chiasma is defined as an exchange of partners in a system of paired chromatids (Janssens, 7). Chiasmata have never been observed in somatic pairing with the exception of an isolated case reported by Kaufmann (8) in *Drosophila melanogaster*. The present work includes a study of somatic pairing induced by heat treatment of barley and a re-examination of the somatic pairing obtained by Kemp (9) in peas (*Pisum sativum*) treated with chloral hydrate.

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Methods and Germination Data

The technique employed to induce the association of chromosomes in the root tips of O.A.C. 21 barley varied slightly in the four treatments reported. All the treatments, however, involved the germination of the seeds at or near the critical temperature of 35–36° C. The seminal root tips were fixed in La-Cour's 2 B.E., sectioned 13 μ thick, and stained with gentian violet (La-Cour, 11).

In Treatment 1 the apparatus used for germinating the seeds consisted of a constant temperature water bath in which half pint sealers containing the seeds were immersed for the treatment period. The seeds were placed on a layer of sand which was saturated with nutrient solution. Preheated air was drawn continuously through the sealer to provide adequate ventilation. As a precaution against the growth of bacteria and molds all the equipment was steam sterilized and the seed was sterilized for five minutes in a solution of 0.5% mercuric chloride and subsequently washed thoroughly in distilled water. The air supply was also sterilized by bubbling it through a 2% solution of hydrochloric acid containing 5% mercuric chloride. The temperature of the bath was maintained at 35° C. and the treatment lasted for 96 hours. At the end of this period 38% of the seeds had produced seminal root tips 4 to 12 mm. long.

The same apparatus and germinating temperature were used for Treatment 2, but the seed was not sterilized and cotton batting was used in place of sand. After 42 hours 66% of the seeds had germinated and root tips were collected from 25 seedlings.

In Treatment 3 an attempt was made to stimulate germination under high temperature conditions by bubbling oxygen through the jars. The treatment and results are given in Table I. It was found that the oxygen apparently did stimulate germination to some extent. The treatment temperature varied between 35.5 and 36.0° C.

TABLE I

EFFECT OF OXYGEN ON GERMINATION IN TREATMENT 3

Treatment	Duration of treatment, hr.	Germination, %
Increased oxygen	26	54
Normal aeration	26	46
Increased oxygen	36	59
Normal aeration	36	31

A Hearson germinator was used instead of a water bath in Treatment 4. The seed was placed on absorbent cotton in crystallizing dishes and the absorbent cotton was kept damp with nutrient solution. Four lots of 100 seeds each were germinated for 41 hours at 35.5–36° C. They were then removed from the germinator and allowed to grow at room temperature. Root tips were fixed at 0, 12, 24 and 48 hours after removal from the germinator. The percentage germination for the four lots was 57, 55, 37 and 28. This wide variation was thought to be the result of the space variation of the temperature in the germinator. It was extremely difficult to duplicate germination results at these critical temperatures since the slightest temperature variation caused very wide fluctuations in germination percentage.

The method employed in the treatment of *Pisum sativum* with chloral hydrate was similar to that used by Kemp (9). Arthur peas were germinated in a Hearson germinator at 18° C. and after four days, 90% of them had germinated. One hundred and twenty uniform seedlings were selected and grown in nutrient solution until they had attained an average height of 2½ inches. The roots of 35 of these plants were then washed carefully to remove the nutrient solution, and immersed in a 0.75% solution of chloral hydrate for one hour. After treatment the roots were again washed in six changes of water for one hour before being replaced in the nutrient solution. A single root tip was taken from each seedling 0, 24 and 72 hours after treatment and fixed for cytological examination.

The plants grew vigorously after being removed from the chloral hydrate and no external indications of permanent injury were noticeable.

Chromosome Associations in Root Tips of Heat Treated Barley

Cytological preparations of root tips from 20 seedlings of Treatment 1 were examined. The high temperature had checked growth to such an extent that division figures were found in root tips of only nine of these seedlings. The division figures were relatively normal in so far as there was no clumping of the chromatin or other definite evidences of degeneration such as observed after treatment with chloral hydrate. A complete analysis was made on all the division figures observed and the resulting data are shown in Table II.

Twenty-five per cent of the nuclei contained one or more of the abnormalities indicated in the table. In addition to associations of two chromosomes and fragments there was a large number of tetraploid nuclei. The latter seemed to be scattered throughout the meristematic region of the root tip and large tetraploid sectors were never observed. This suggests that they had originated in recent cell divisions and this opinion is confirmed by the juxtaposition of what are obviously daughter chromosomes in these tetraploid cells (Fig. 23). It is believed that the tetraploid cells arose through failure of the chromosomes to separate after splitting, and consequently the daughter chromosomes have maintained their juxtaposition. This paired condition would hardly be maintained in subsequent divisions.

In Treatment 2, 66% of the seed germinated, which was 28% more than in Treatment 1, and, as might be expected, there were fewer abnormalities. Similar results were obtained in Treatment 3 except that a larger number of tetraploid nuclei were observed. The number of nuclei analyzed in this treatment was too small to show any differences between the lots which had been germinated under normal aeration and with increased oxygen concentration.

TABLE II

COMPLETE ANALYSIS OF METAPHASE NUCLEI IN HEAT TREATED BARLEY

Treatment	Fixation	Nuclei analyzed	Normal nuclei		Chromosome association			Fragments			Chromosomes with one broken chromatid	Tetraploid nuclei
			No.	%	Two	One and one-half	Three	Broken at attachment constriction	Small	Large		
1	Immediately after treatment	144	108	75.0	13	0	0	7	1	1	1	20
2	Immediately after treatment	93	85	91.4	4	0	0	2	1	0	0	1
3	Immediately after treatment	60	42	70.0	8	1	0	1	0	0	0	9
4	Immediately after treatment	0										
4	8 hr. after treatment	233	148	63.5	33	8	1	210	5	2	4	1
4	24 hr. after treatment	141	112	79.4	13	8	1	146	2	0	3	0
4	48 hr. after treatment	114	110	96.5	0	0	0	2	5	0	0	0
Totals		785	605	—	71	17	2	368	14	3	8	31

The object of Treatment 4 was to ascertain the frequency of the cytological abnormalities after various normal growth periods subsequent to the completion of the treatment. The treatment was so severe that there were no division figures in the root tips taken immediately on removal from the treatment. Eight hours after treatment the division figures were again very abundant, and 36.5% of the nuclei analyzed contained various chromosomal abnormalities in the proportions recorded in Table II. Twenty-four hours after treatment 20.6% of the nuclei analyzed were abnormal, while after 48 hours only 3.5% were abnormal. This rapid disappearance of these abnormalities is in accord with the observations on the disappearance of chromosomal abnormalities in seedlings grown from aged and heat treated seed (16).

The mode of origin of the paired associations can be deduced from observations at prophase and metaphase stages, as well as from the occurrence in the same nucleus of fragments, chromosomes with broken chromatids, and associations of 1½ chromosomes. The majority of the pairs have a chiasma located at or near the attachment constriction (Figs. 2, 9, 10, 17 and 21). This suggests that the breaks and re-attachments of the chromatids to form these chiasmata took place in the attachment constriction region. This conclusion is substantiated by the observation that chromatid fracture occurred over twenty times more frequently in this region of the chromosome than in any other. In Treatment 4, examined eight hours after treatment, there were 210 fragments of this kind in 233 nuclei. Furthermore, these fragments were often found in the same nucleus with associations of two and three chromosomes (Figs. 3 and 7). Chromosomes with one broken chromatid were also observed at prophase and metaphase as shown in Figs. 24, 25 and 26.

It was not possible to determine the exact time of occurrence of the chromatid fractures, but it must have been subsequent to the splitting of the chromosome and prior to the prophase stage at which fractures have been observed. Typical examples of the earliest stages at which pairs have been observed are shown in Figs. 27 to 30. It will be noted in Fig. 29 that two of the chromatid ends are not yet united. Whether or not the broken ends of chromatids can be reunited at any stage of nuclear division has not yet been determined.

The formation of the paired associations can be explained as follows: The high-temperature growth condition causes numerous chromatid breaks to occur, mainly in the attachment regions. The fractured ends of the chromatids evidently possess an unsatisfied attraction or bond and will unite with the fractured ends of any other chromatids lying in the vicinity. Huskins and Hunter (4) came to a similar conclusion from observations on X-rayed microspores of *Trillium erectum*. If this unsatisfied attraction did not exist it would be expected that the broken ends might also unite with the ends of normal chromosomes, but configurations which would indicate that this had taken place were never observed. When two chromosomes, each with one broken chromatid in the attachment region, lie close together in prophase, they would probably unite to form one of the types of paired associations observed.

The paired associations were classified in an attempt to determine whether the re-attachment of the broken chromatids was at random or whether there was any special attraction between the broken ends of chromatids of homologues fractured in the same region. Unfortunately it was impossible to identify positively all the chromosomes. The heteromorphic class (Table III) contains all the pairs in which the chromosomes are obviously non-homologous or which have united to form a configuration with unequal arms. Examples of heteromorphic pairs are shown in Figs. 3, 7 and 17. The last named figure is positive proof of non-homology since one of the chromosomes with a trabant was found unpaired and the other was included in a pair. The same kind of proof is shown in the association of three in Fig. 4, and the association of 1½ in Fig. 18. The occurrence of associations of three (Figs. 3 and 4), regardless of their morphological peculiarities, is in itself a proof of the union of broken ends of non-homologous chromatids, since there are only two homologous chromosomes in this diploid species. If heteromorphic pairs occur in the primordia of the spike, a further proof of non-homologous crossing-over should be found in the heterotypic division where rings of four chromosomes should occasionally be observed. McClintock (12) uses a similar proof of segmental interchange in maize.

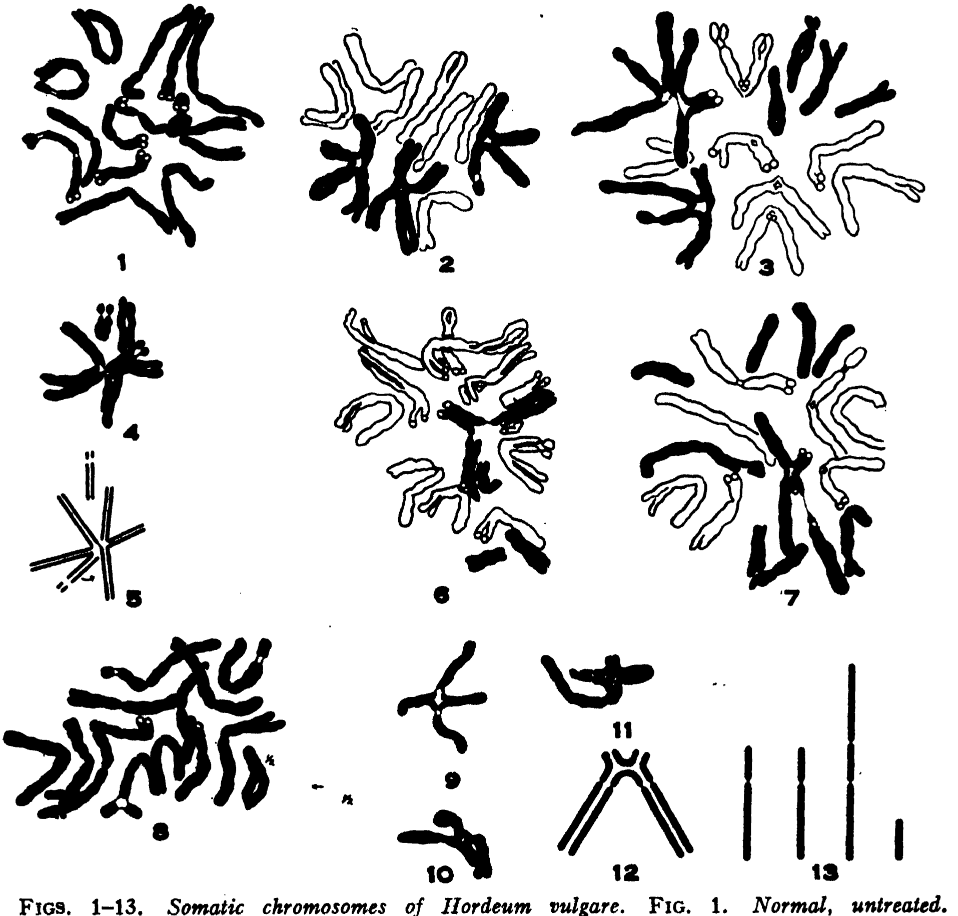
The homomorphic class in Table III includes associations of similar appearing chromosomes which may or may not be homologous. This class was sub-divided according to the way in which the fractured ends were united to form chiasmata. The normal type as shown in Figs. 9 and 10, and Plate I, Fig. A, is the result of the union of fractured ends to form a chiasma similar to that formed at meiosis. The abnormal type of homomorphic bivalent is shown in Figs. 11 to 16. The identification of this type was most positive

TABLE III

TYPES OF PAIRED ASSOCIATIONS AT METAPHASE IN HEAT-TREATED BARLEY

Treatment	Fixation	Homomorphic		Heteromorphic	Unclassifiable	Total
		Abnormal	Normal			
1	Immediately after treatment	3	4	2	5	14
2	Immediately after treatment	0	0	2	2	4
3	Immediately after treatment	4	0	1	3	8
4	Immediately after treatment	0	0	0	0	0
4	8 hr. after treatment	13	9	10	17	49
4	24 hr. after treatment	1	3	7	7	18
4	48 hr. after treatment	0	0	0	0	0
Totals		21	16	22	34	93

in pairs with subterminal chiasmata. Instead of the diagonal ends of the broken chromatids uniting as must be the case in normal meiosis, the adjacent homologous or homomorphic ends united. This is evidently what happened in the configuration shown in Fig. 11. The interpretation of the chromatid arrangement is shown in Fig. 12, and the expected constitution of the daughter chromosomes is shown in Fig. 13. One of the daughter chromosomes would be a small fragment without any attachment constriction and the halves of this fragment would likely be homologous. The long daughter chromosome would have two attachment constrictions and again the halves would likely

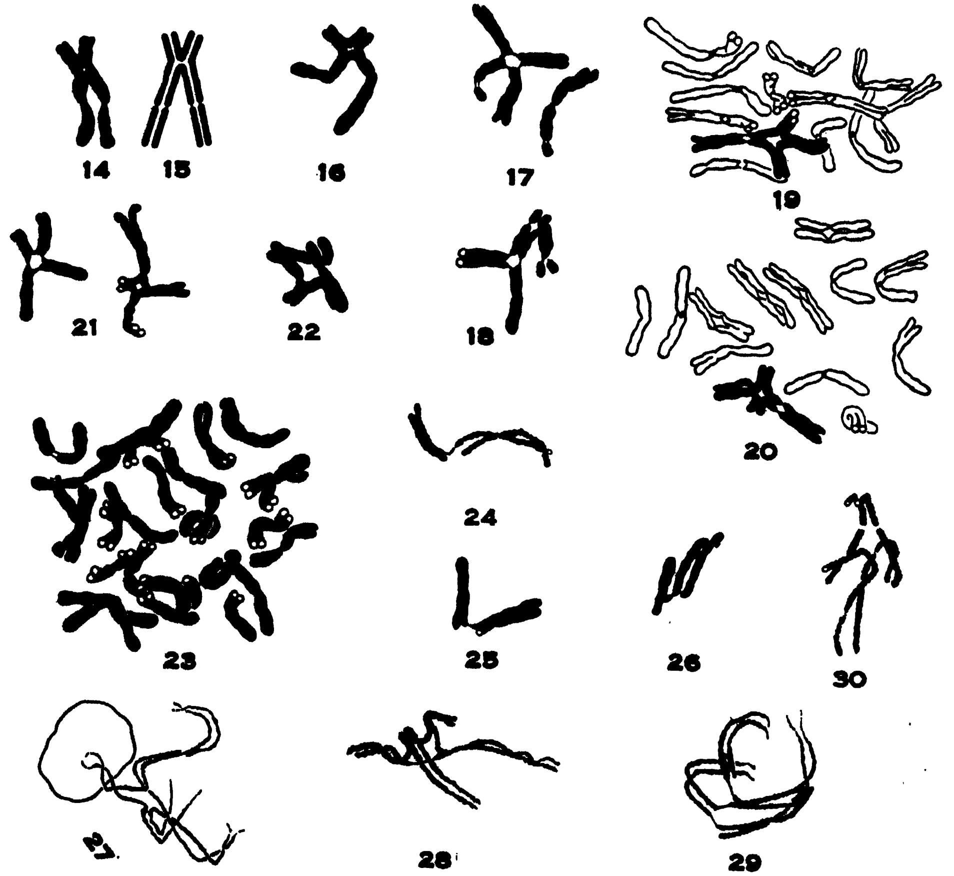


FIGS. 1–13. *Somatic chromosomes of Hordeum vulgare.* FIG. 1. *Normal, untreated.* FIG. 2. *Three pairs of chromosomes.* FIG. 3. *Associations of two and three, four half chromosomes.* FIG. 4. *Chromosome with trabant included in association of three; half chromosome with trabant unattached.* FIG. 5. *Diagram of Fig. 4.* FIG. 6. *Associations of one and one-half, three half chromosomes.* FIG. 7. *One pair with broken chromatid, one chromosome with broken chromatid, one chromosome with abnormally short arm, six half chromosomes.* FIG. 8. *Double trabant, two half chromosomes.* FIG. 9. *Homomorphic normal pair.* FIG. 10. *Homomorphic normal pair.* FIG. 11. *Homomorphic abnormal pair.* FIG. 12. *Diagram of Fig. 11.* FIG. 13. *Diagram of the separate chromatids of Fig. 11.*

Magnifications: Figs. 1, 10, 19, 20, 21, 23, × *2250. Figs. 11, 14, 16,* × *2750. Remainder* × *2400.*

be homologous. It would be deficient, however, by the region included in the small chromosome. The two other daughter chromosomes would be normal. The above case is an excellent illustration of how duplications and deficiencies can arise in somatic tissue.

The data resulting from a classification of the paired associations are given in Table III. There is a total of 37 homomorphic, 22 heteromorphic and 34 unclassifiable pairs. If homologous chromosomes could have been accurately detected and the union of broken chromatids was purely at random, there should be six times as many heteromorphic as homomorphic pairs. However, the similarity of many of the chromosomes in the complement may have resulted in many of the non-homologous pairs being classified as homomorphic.



FIGS. 14–29. *Somatic chromosomes of Hordeum vulgare.* FIG. 14. *Homomorphic abnormal pair.* FIG. 15. *Diagram of Fig. 14.* FIG. 16. *Homomorphic abnormal pair.* FIG. 17. *Heteromorphic pair plus chromosome homologous to one included in pair.* FIG. 18. *Association of one and one-half plus homologous half chromosome.* FIG. 19. *Unclassifiable pair.* FIG. 20. *Unclassifiable pair.* FIG. 21. *Two unclassifiable pairs.* FIG. 22. *Pair at early anaphase.* FIG. 23. *Tetraploid nucleus.* FIG. 24. *Prophase chromosome with broken chromatid.* FIG. 25. *Metaphase chromosome with broken chromatid.* FIG. 26. *Metaphase chromosome with broken chromatid.* FIG. 27. *Pair at prophase, outline of nucleolus.* FIG. 28. *Pair at prophase.* FIG. 29. *Pair at prophase with two unattached ends of chromatids.* FIG. 30. *Pair at prophase.* *Magnifications: Figs. 19, 20, 21, 23, 30,* × *2250. Figs. 14, 16,* × *2750. Remainder* ×*2400.*

This would reduce the proportion of heteromorphic pairs. In spite of this difficulty of classification the positive examples combined with the general data make it apparent that pairing of the chromosomes is not the result of any specific attraction between homologues but rather a haphazard or random union of broken chromatids. This is further emphasized by the observed proportion of 16 normal to 21 abnormal homomorphic pairs and also by the fact that homomorphic or homologous chromosomes do not show any tendency to pair unless they are united by chiasmata.

Associations of one and one-half chromosomes are shown in Figs. 6 and 18. These probably were produced either through the union of a chromosome possessing one broken chromatid with a half chromosome, or through the attachment of the broken chromatids of three half chromosomes. These associations occurred with about ¼ of the frequency of the paired condition.

Chromatid breaks are not confined to the attachment constriction region as shown by the double trabant in Fig. 8, the broken chromatid in the sub-terminal region of one of the chromosomes in Fig. 7 and the sub-terminal chiasmata in Figs. 11, 14 and 16. The possibility should also be considered that many more breaks than are shown at metaphase may occur throughout the length of the chromosome and may be covered up by the immediate reunion of broken chromatids. There is also great likelihood of many of the halves of non-homologous chromosomes being united to form normal appearing chromosomes. The reuniting of chromosome halves, whether they are homologous or non-homologous, may account for the absence of fractured chromosomes 48 hours after treatment.

A number of different re-arrangements of chromosome segments have been found in maize (Rhoades and McClintock, 17). These were believed to have resulted from a two-by-two union of broken ends of chromosomes. The broken ends of barley chromosomes may also unite in this manner but the evidence shows that frequently only one of the two chromatids at a particular locus breaks, so that the observed chiasmata are formed on re-attachment with the broken ends of other chromatids. It seems likely that some of the new chromosomal types in maize may have originated in this manner rather than by the method postulated by Rhoades and McClintock.

Chromosome Pairing in Polyploid Nuclei of Peas

The data on the analysis of the metaphase nuclei in root tips of peas treated with chloral hydrate are shown in Table IV. Those specimens that were fixed immediately after treatment with chloral hydrate were very abnormal. Nuclear division was almost completely checked and only in a few root tips was it possible to obtain chromosome counts. In the majority of the cells there was a marked coagulation of the nuclear material so that it often appeared as an amorphous mass. In those in which counts were possible, the chromosomes at metaphase appeared to be shorter and thicker than usual and their margins were abnormally rough. All the nuclei analyzed had nevertheless the normal complement of 14 chromosomes.

TABLE IV

ANALYSIS OF METAPHASE NUCLEI IN PEAS TREATED WITH CHLORAL HYDRATE

Treatment	Fixation	Nuclei analyzed	Normal nuclei	Tetraploid nuclei	Octaploid nuclei
5	Immediately after treatment	25	25		
5	24 hr. after treatment	59	35	19	4
5	72 hr. after treatment	33	13	20	0

After 24 hours' growth subsequent to treatment, an unusually high proportion of the nuclei were in active division and there was no evidence of nuclear coagulation so apparent in the material examined immediately after treatment. A normal nucleus containing 14 chromosomes is shown in Plate I, Fig. F. Polyploid nuclei were very frequent in these root tips, 19 tetraploid and 4 octaploid nuclei being observed. One diploid nucleus was found with two fractured chromosomes, but none of the other abnormalities found in the heat-treated barley were seen. Only a limited number of the apparently normal nuclei were examined.

The root tips examined 72 hours after treatment were very similar to those examined after 24 hours. Twenty tetraploid but no octaploid nuclei were found. Many apparently normal nuclei were observed, but here again only a limited number were analyzed.

Koshuchow (10) found numerous tetraploid areas in the root tips of *Cucumis sativus* L. and *Zea Mays* var. *indenta* which had been subjected to heat and cold treatments. These cells were found only in the oldest part of the periblem and never in the neighborhood of the initial cells, the endodermis, the pericycle or the central cylinder. In the root tips of peas treated with chloral hydrate, the polyploid nuclei were scattered throughout the various tissues of the meristematic region. Large polyploid sections were never observed, but it would not be expected that many divisions could have occurred within 72 hours after treatment.

Excellent evidence was available on the manner in which polyploid nuclei were formed. In many instances division and separation of the chromosomes to the poles appears to proceed normally, but cytokinesis is apparently checked and no dividing wall is laid down. An example of this is shown in Plate I, Fig. C, in which there is a cell containing two nuclei, each at the metaphase stage. If a restitution nucleus is not formed immediately, these chromosomes may split again, resulting in a tetrapolar spindle of which an example is shown in Figs. 33 and 34. This cell contains four nuclei, each with 14 chromosomes. Nemec (15) found multipolar spindles in giant cells of galls caused by *Heterodera radicicola* on the roots of sugar beets. He believed that these multipolar spindles resulted in a reduction in the chromosome number in polyploid nuclei and that this accounted for their disappearance in subsequent

PLATE I

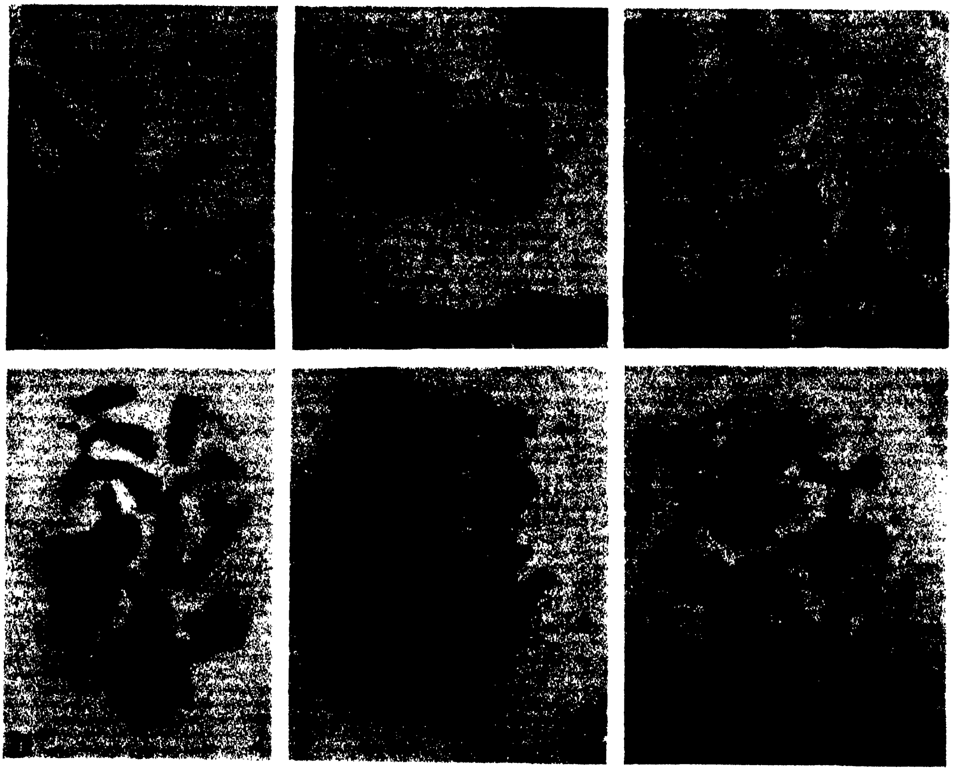
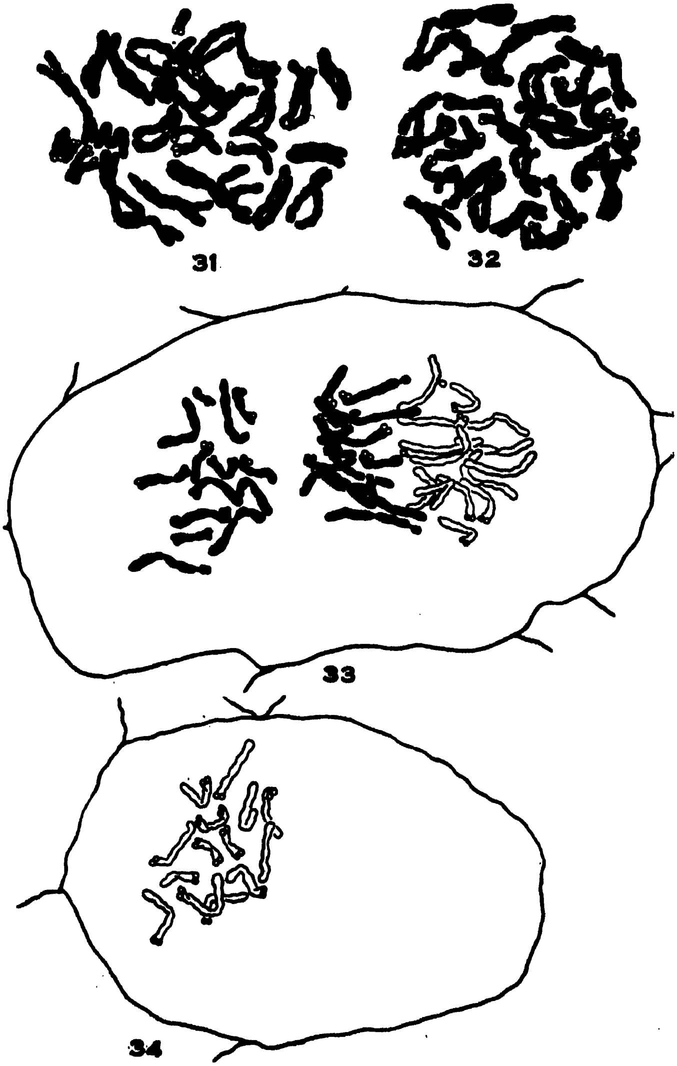


FIG. A. *Somatic association of two chromosomes in Hordeum vulgare.* ×*5000.* FIG. B. *Prophase of mitosis in Hordeum vulgare showing a partial chiasma.* FIG. C. *Two nuclei at metaphase in the same cell of Pisum sativum.* ×*1500.* FIG. D. *Octaploid cell in Pisum sativum showing chromosome pairs without chiasmata.* ×*3250.* FIG. E. *Focus at different level in same cell as shown in Fig. D.* ×*3250.* FIG. F. *Normal nucleus of Pisum sativum.* ×*3250.*

divisions. Nemec's findings are in contrast to the interpretation of the multipolar spindles found in *Pisum sativum* which in the absence of normal cytokinesis are believed to give rise to the polyploid nuclei. It is suggested that the latter interpretation may be the true explanation of the origin of the multinucleate condition in the galls of the sugar beet. This would account for the occurrence of polyploid cells if restitution nuclei were formed, but not for their disappearance.



FIGS. 31–34. *Somatic chromosomes of Pisum sativum.* FIGS. 31 AND 32. *Octaploid nuclei showing association of the chromosomes in pairs without chiasmata.* FIG. 33. *Portion of giant cell containing three groups of 14 chromosomes.* FIG. 34. *Remaining portion of the above cell containing the fourth group of 14 chromosomes. Magnifications:* × *2400.*

It frequently happens that there is a faulty separation of the daughter chromosomes at anaphase. In these cases the chromosomes remain in a paired arrangement as shown in the octaploid nuclei in Figs. 31 and 32, and Plate I, Figs. D and E. This is undoubtedly the type of pairing reported by Kemp (9) in peas, and Huskins and Smith (5) in sorghum. It is apparently not due to any attraction between homologues, but rather to a failure of the spindle mechanism. There is a further argument against this paired arrangement in peas being due to homologous attraction in that it is never observed in diploid nuclei, nor are associations of four and eight chromosomes observed in tetraploid and octaploid nuclei. Pairing of homologous chromosomes in somatic diploid nuclei has, however, been reported by Gates (2) in *Oenothera*, by Janaki-Ammal (6) in *Nicandra physaloides* and by Watkins (19) in Yucca as well as many instances in *Diptera* by Metz (13, 14) and others.

It is readily understandable how some of the early workers who observed the type of paired arrangement found in the polyploid cells of peas should suggest its relationship to normal meiotic pairing. The importance of chiasmata in retaining the paired association from diplotene to metaphase of meiosis was not appreciated at that time. Critical examination of the somatic pairs in *Pisum sativum* which appear superficially much like true bivalents (Plate I, Figs. D and E) reveals that chiasmata are not present. This evidence is presented to show especially the contrast between the paired association without chiasmata found in the peas treated with chloral hydrate and the paired association involving chiasmata found in the heat-treated barley.

Discussion

The chromosome associations found in the root tips of the heat-treated barley clearly demonstrate how chromatid interchange or crossing-over can be accomplished in somatic tissue. There can be no doubt that the chiasmata observed in this material are the result of breaks and reattachments of chromatids and that the presence of the chiasmata is substantial proof that chromatid interchange has taken place. The homomorphic abnormal pairs shown in Figs. 11, 14 and 16 illustrate this point. The abnormally short chromatids lacking attachment constrictions and the abnormally long chromatids with two attachment constrictions could hardly have arisen in any other manner than by chiasma formation involving chromatid break and reattachment.

Mottling, unstable translocations, and duplications in *Drosophila melanogaster* have been recently explained by Stern (18) on the basis of somatic crossing-over and segregation. The mechanism by means of which this crossing-over and segregation could be accomplished in somatic tissue has never been satisfactorily demonstrated. The only known instance of chiasma formation in somatic tissue was reported by Kaufmann (8) in the ganglia cells of larvae of *Drosophila melanogaster*. He found that the four chromatids of the tightly appressed homologues opened out into two planes giving distinct chiasma-like configurations, and that the pairing of the chromosomes reached its maximum expression during late prophase. These paired chromosomes began to separate at this stage and at metaphase they were not in contact. Kaufmann did not consider that the presence of these chiasmata was sufficient proof that crossing-over had taken place. His observations, therefore, do not give a satisfactory explanation of somatic segregation and crossing-over.

The elimination or duplication of a part of a chromosome could result in sectorial effects similar to that expected from somatic segregation. Both deficiencies and duplications are involved in the chromatids of the homomorphic abnormal pairs shown in Figs. 11, 14 and 16. If in anaphase separation of the chromatids in Fig. 11, the longest chromatid with the two attachment constrictions were included in one daughter nucleus and the remaining three chromatids in the other, then the nucleus containing the abnormally long chromosome would be deficient by two short homologous portions. The missing portions would constitute the short chromosome lacking the attachment constriction. If such a nucleus were viable it would likely produce an altered character expression which might be interpreted as being the result of gene mutation or somatic segregation. These possibilities are pointed out to indicate the difficulties in determining the cause of sectorial chimeras which superficially appear to be the result of somatic segregation. Such a case was reported in the Bartlett pear by Gardner, Crist and Gibson (1).

Horlacher and Killough (3) observed cotton bolls giving 100% normal leaf progeny on plants grown from heterozygous seed which had been subjected to X-ray treatment. One of their two possible explanations of this phenomenon was that the chromosome carrying the recessive gene was deleted by the X-ray treatment and that all the functional gametes possessed only the gene for normal leaf. The observations on the heat-treated barley suggest one mechanism by which deletions of parts of chromosomes could occur.

Chiasma formation in somatic bivalents of barley differ from those found by Kaufmann in *Drosophila* in that the homologues of the latter are closely paired in all cases and chiasmata always unite homologous chromosomes. In the heat-treated barley there is no evident attraction between homologous chromosomes. The chromatid breaks apparently leave the fractured ends with an unsatisfied attraction which results in their being united at random with any others located in the vicinity. Under these conditions chromatid interchange between non-homologous chromosomes and the consequent formation of new linkage relationships would be of frequent occurrence. Heat treatments may therefore be useful in breaking the linkage between desirable and undesirable plant characters or in the formation of a new linkage of genes for desirable characters. However, it is not yet known whether chromatid interchange can be induced by heat treatments in species other than *Hordeum vulgare*.

Acknowledgment

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THE BREEDING OF DISEASE-RESISTANT SMOOTH-AWNED VARIETIES OF BARLEY[1]

BY W. H. JOHNSTON[2] AND O. S. AAMODT[3]

Abstract

Barbing of awn, earliness of heading, height of plant and disease reaction were investigated in reciprocal crosses between Glabron and Trebi. Barbing of awn was also studied in reciprocal crosses between Velvet and Trebi. Rough-, intermediate- and smooth-awned plants were obtained in the ratio of 12:3:1 in the F_2. F_3 studies confirmed the F_2 findings that two factors, explained on the basis of epistasis, governed barbing of awns.

The inheritance of the characters, earliness of heading and height of plant could best be explained by assuming polymeric factors. Both these characters were greatly influenced by the environment.

In the genetic studies of stripe reaction Trebi proved highly resistant, and Glabron moderately resistant, while the progeny of the cross between them generally resembled the Trebi parent. No evidence of transgressive reaction for greater resistance was shown. The results indicate that the method of floral inoculation used was not altogether satisfactory.

A small positive correlation was found between percentage stripe infection and mean height of plant, whereas no significant correlation was disclosed between stripe reaction and either mean number of days from emergence to heading or barbing of awns. A high degree of association was found between mean height of plant and mean number of days from emergence to heading.

The status of the loose smut disease of barley in relation to the breeding of new resistant smooth-awned varieties is discussed.

Introduction

When a character which may assume the role of a limiting factor is overlooked in a breeding project, the resulting improved variety is likely at any time to become a failure as far as the producer is concerned. This principle is particularly applicable to the problem of breeding for disease resistance in field crop work. In the breeding of smooth-awned barleys it is important that such a desirable character as smooth awn be associated with the equally desirable one of disease resistance.

Certain progress has already been made in this direction. The Minnesota Agricultural Experiment Station has introduced several smooth-awned varieties resistant to the spot blotch disease (*Helminthosporium sativum*) (19, 20). The Wisconsin Agricultural Experiment Station has been concentrating on the development of new smooth-awned varieties possessing resistance to the stripe disease, *Helminthosporium gramineum* (38). Griffee (12) studied the reaction of barley hybrids to *H. sativum* in crosses between rough- and smooth-awned parents. Isenbeck (21) investigated stripe reaction in crosses involving Velvet as one parent. Recently Powers and Hines (31) studied the resistance to three forms of stem rust in barley crosses between Peatland (rough-awned,

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resistant) and Glabron (smooth-awned, susceptible). They concluded that no difficulty should be encountered in obtaining smooth-awned selections, resistant to the forms of rust used in their investigation.

In 1929, a barley breeding project was begun at the University of Alberta, Edmonton, for the purpose of developing new, smooth-awned, disease-resistant, barley varieties suitable for Alberta conditions. Attention was confined mainly to covered smut and stripe, although some attention was given to loose smut. During the course of this investigation genetic data were also compiled on barbing of awn, earliness of heading and height of plant. The results obtained regarding the covered smut phase of this investigation have been reported by Johnston (23), and Aamodt and Johnston (2).

Materials and Methods

The barley varieties used as parents were two smooth-awned types, Glabron and Velvet, and a rough-awned type, Trebi. Reciprocal crosses were made between Trebi and each of the smooth-awned varieties.

Trebi is a six-rowed, rough-awned variety originating as a pure line selection in 1907 in the co-operative breeding experiments conducted by the United States Department of Agriculture and the Minnesota Agricultural Experiment Station at St. Paul (17). Trebi is mid-late in maturity, mid-tall, susceptible to *Helminthosporium sativum*, moderately susceptible to *Ustilago hordei* and resistant to *Ustilago nuda* and *Helminthosporium gramineum*.

Glabron and Velvet are six-rowed, smooth-awned varieties developed at the Minnesota Agricultural Experiment Station (19). Glabron was developed from a backcross between Smooth Awn (a selection from a cross between Lion and Manchuria) and Manchuria, whereas Velvet resulted from a cross between Smooth Awn and Luth. Both Glabron and Velvet possess a good length of straw and are mid-late in maturity. A summary of the agronomic performance of these two varieties in Alberta for the five year period 1930-34 has recently been made by Aamodt and Johnston (1). These varieties are resistant to *U. hordei* and *H. sativum* and susceptible to *U. nuda*. Velvet is highly susceptible to *H. gramineum*, whereas Glabron is moderately so.

The crosses involving Trebi and Velvet were used only to study the inheritance of barbing of awn in the F_2. This character was also studied in the F_1, F_2 and F_3 of crosses between Glabron and Trebi. Earliness of heading, height of plant, and disease reaction were studied in the F_3 of the last-named cross. The F_3 lines were grown in ten-foot rows with approximately 50 seeds per row. Parental check rows were sown at frequent intervals.

Barbing of Awns

The results of the more important studies on the inheritance of barbing of awns are given in Table I. There is a considerable difference in the mode of inheritance of barbing of awns reported. Since different parental material was used by the various investigators, the differences in results obtained and the conclusions drawn are not unexpected.

TABLE I

SUMMARY OF RESULTS OF STUDIES BY A NUMBER OF INVESTIGATORS ON THE INHERITANCE OF BARBING OF AWN IN BARLEY

Investigator	Materials	Behavior
Harlan (13)	Several crosses	Rough awn dominant, single factor.
Vavilov (50)	Several crosses	5–6 pairs of factors.
Hayes *et al.* (20)	Lion × Manchuria	One main factor, plus modifiers.
Griffee (12)	Lion × Svanhals	Two factors explained on basis of epistasis.
Sigfusson (39)	Bearer × Lion Chinese × Lion	Two complementary factors.
David (7)	Glabron × Trebi Velvet × Trebi	One main factor for smooth awn and one main factor inhibiting smooth awn.
Robertson, Deming and Koonce (33)	Coast × Lion	Two factors explained on basis of epistasis.
Wexelsen (52)	Machine × Smooth Awn 4252	Single factor.

METHODS AND EXPERIMENTAL RESULTS

The degree of barbing of the awns was estimated simply by passing the awns between the fingers. This simple and rapid method was adopted rather than the more complex index system, because of the large number of individuals in the F_2 population to be studied and because it was thought that, in the event of using an arbitrary index, the true phenotypic classes might be overlooked. An examination of a large number of plants of the smooth-awned parents showed a considerable variation in the degree of barbing of the awns. This varied from a few barbs at the extreme tips of the awns to barbs extending one-quarter of the length of the awns.

The F_1 of all the crossses studied were rough-awned. It was a comparatively simple matter to separate the F_2 plants into two main groups: those with completely rough awns and those with awns exhibiting some degree of smoothness. Greater diffiiculty was experienced in establishing classes within these two groups. After considerable study the following awn classes were decided upon:

1. Rough	rough
2. Near-rough	
3. Smooth-base	intermediate
4. Near-smooth	
5. Smooth	smooth

The first two classes have fully barbed awns, but the barbs of the second class are much less scabrous than those of the first. The awns of Class 3 have prominent barbs extending along 50–75% of their length, whereas in Class 4 the barbs seldom extend over 50% of the length of the awn and are

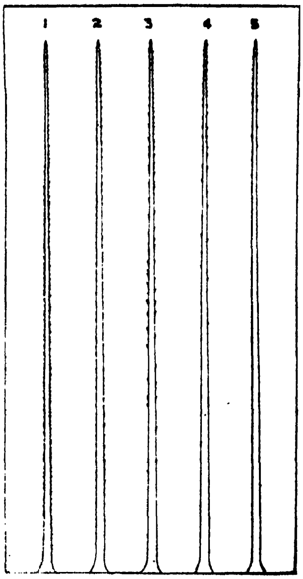

FIG. 1. *Diagrammatic length-wise section of average awn of each of five awn classes: (1) rough, (2) near-rough, (3) smooth-base, (4) near-smooth, and (5) smooth; used in classification of F_2 of reciprocal crosses of Glabron and Trebi and Velvet and Trebi.*

not as scabrous as those of Class 3. Class 5 reflects the smooth awn of the parental type. An average awn of each of the five classes is illustrated diagrammatically in Fig. 1.

Considerable variation was observed within the two rough-awned and two intermediate classes. Since environmental factors might be responsible for these variations, the segregation of F_2 plants for barbing of awn in the crosses studied has been based upon three phenotypes: rough, intermediate and smooth. The near-rough class was combined with the rough class, while the smooth-base and near-smooth classes were grouped to form a single intermediate class. This grouping suggested a 12:3:1 ratio of rough : intermediate : smooth-awned plants. The actual segregations as compared with the theoretical 12:3:1 are given in Tables II and III. The fit of the actual to the theoretical in the crosses involving Trebi and Glabron (Table II) is fairly good in some cases, but owing to the tendency to place a number of intermediate plants in the smooth-awned class, the total population shows a poor fit to the theoretical.

On the other hand, the reciprocal crosses of Trebi and Velvet, together with their total, show good fits to the theoretical (Table III). This may be explained by the fact that, as this cross was studied last, a greater familiarity with the three phenotypes had been acquired.

The F_2 data just presented suggest a two factor difference for barbing of awn. The segregation can be explained on the basis of epistasis; R is the main factor for barbing of awn; S is hypostatic to R and in the absence of R produces the intermediate condition, while the double recessive produces the smooth-awned type. On the basis of this hypothesis the F_2 plants, when tested in the F_3, should breed as follows:

TABLE II

NUMBERS OF ROUGH-, INTERMEDIATE- AND SMOOTH-AWNED PLANTS IN THE F_2 GENERATION OF RECIPROCAL CROSSES BETWEEN GLABRON AND TREBI, AS COMPARED WITH THE THEORETICAL 12:3:1 RATIO

Cross	No.	Actual			Theoretical			χ^2	P
		Rough	Intermediate	Smooth	Rough	Intermediate	Smooth		
Glabron × Trebi	6	111	21	7	104.28	26.07	8.69	1.75	0.43
	11	133	21	18	129.00	32.25	10.75	9.64	0.01
	12	170	32	16	163.50	40.88	13.63	2.60	0.28
	13	121	29	18	126.00	31.50	10.50	5.71	0.06
Trebi × Glabron	19	79	24	13	87.00	21.75	7.25	5.64	0.06
	20	158	33	14	153.75	38.44	12.81	1.00	0.61
	21	95	20	5	90.00	22.50	7.50	0.84	>0.61
	23	83	13	8	78.00	19.50	6.50	2.83	0.25
	45	136	22	10	126.00	31.50	10.50	3.68	0.16
Total		1086	215	109	1057.56	264.39	88.13	14.93	0.001

TABLE III

NUMBERS OF ROUGH-, INTERMEDIATE- AND SMOOTH-AWNED PLANTS IN F_2 GENERATION OF RECIPROCAL CROSSES BETWEEN VELVET AND TREBI, AS COMPARED WITH THE THEORETICAL 12:3:1 RATIO

Cross	No.	Actual			Theoretical			χ^2	P
		Rough	Intermediate	Smooth	Rough	Intermediate	Smooth		
Trebi × Velvet	116	59	15	8	61.50	15.38	5.13	1.72	0.44
	123	160	30	12	151.50	37.88	12.63	2.15	0.35
	124	155	37	16	156.00	39.00	13.00	less than 1	>0.61
	133	186	50	19	191.38	47.82	15.94	1.73	0.43
Velvet × Trebi	137	183	45	16	183.00	45.75	15.25	less than 1	>0.61
	146	123	22	6	113.16	28.29	9.44	3.51	0.18
	148	188	41	14	182.28	45.57	15.19	less than 1	>0.61
	151	114	23	8	108.72	27.18	9.06	1.02	0.60
Total		1168	263	99	1147.56	286.89	95.63	2.47	0.30

	F_2 *genotype*	F_3 *behavior*
rough	1 *RRSS* 2 *RRSs* 1 *RRss*	4 breeding true for rough.
	4 *RrSs*	4 segregating in the ratio 12:3:1 for rough : intermediate : smooth.
	2 *RrSS*	2 segregating 3 rough : 1 intermediate.
	2 *Rrss*	2 segregating 3 rough : 1 smooth.
intermediate	1 *rrSS*	1 breeding true for intermediate.
	2 *rrSs*	2 segregating 3 intermediate : 1 smooth.
smooth	1 *rrss*	1 breeding true for smoothness.

Three crosses involving Trebi and Glabron were given further study in the F_3. The behavior of F_3 lines from rough-awned F_2 plants is shown in Table IV.

TABLE IV

THE BEHAVIOR OF F_3 LINES FROM ROUGH-AWNED F_2 PLANTS COMPARED WITH THE THEORETICAL RATIO 4:4:2:2

Cross	No.	Non-seg.		Seg. 12:3:1		Seg. 3 rough: 1 int.-smooth		Seg. 3 rough: 1 smooth		χ^2	P
		Act.*	Theor.	Act.*	Theor.	Act.*	Theor.	Act.*	Theor.		
Glabron × Trebi	12	63	47.67	29	47.67	32	23.83	19	23.83	15.960	0.001
Trebi × Glabron	21	24	26.67	25	26.67	17	13.33	14	13.33	1.42	0.71
Trebi × Glabron	45	39	36.00	33	36.00	22	18.00	14	18.00	2.28	0.52
Total of Crosses 21 and 45		63	62.67	58	62.67	39	31.33	28	31.33	2.58	0.47

Corrected numbers based on F_3 behavior.

The fits are remarkably close to the theoretical in Crosses 21 and 45, but a wide deviation occurs in the case of Cross 12. It will be seen that the homozygous rough-awned lines are too numerous, whereas those segregating in the ratio of 12:3:1 are too few in number. This poor fit may be explained only as a chance variation as it would seem very unlikely that lines segregating for any degree of smoothness would be placed in the homozygous rough-awned class. The actual numbers of different segregates in F_3 lines derived from rough-awned F_2 plants show close approximations to the expected.

In Table V is shown a summary of the lines segregating for rough- : intermediate- : smooth-awned plants as compared with the theoretical 12:3:1. It is evident that good fits to the theoretical exist in all three crosses.

TABLE V

SUMMARY OF THE RATIOS OF ROUGH- : INTERMEDIATE- : SMOOTH-AWNED PLANTS IN F_3 SEGREGATING LINES, COMPARED WITH THE THEORETICAL 12:3:1

Cross	No.	—	Rough	Intermediate	Smooth	χ^2	P
Glabron × Trebi	12	Act. Theor.	879 892.50	226 223.13	85 74.38	1.76	0.47
Trebi × Glabron	21	Act. Theor.	800 789.00	182 197.25	70 67.25	1.61	0.46
Trebi × Glabron	45	Act. Theor.	1014 1020.75	258 255.19	89 85.06	0.27	>0.61

A summary of the numbers of rough- to intermediate-awned plants in lines segregating 3:1 as compared with the theoretical numbers is given in Table VI. Satisfactory fits to the theoretical exist in the cases of Crosses 21 and 45, but only a fair approximation in the case of Cross 12. It will be noted in all three crosses that there are too many rough-awned plants at the expense of the intermediate. This discrepancy is particularly great in Cross 12.

TABLE VI

SUMMARY OF THE RATIOS OF ROUGH- : INTERMEDIATE-AWNED PLANTS IN F_3 SEGREGATING LINES, COMPARED WITH THE THEORETICAL 3:1

Cross	No.	—	Rough	Intermediate	$\frac{\text{Dev.}}{\text{P.E.}}$	Odds
Glabron × Trebi	12	Act. Theor.	969 927.75	268 309.25	4.02	142.26 : 1
Trebi × Glabron	21	Act. Theor.	551 537.00	165 179.00	1.77	3.45 : 1
Trebi × Glabron	45	Act. Theor.	674 655.50	200 218.50	2.14	5.38 : 1

A summary of the ratios of rough- to smooth-awned plants in segregating lines as compared with a theoretical 3:1 revealed excellent fits in all three crosses (Table VII).

TABLE VII

SUMMARY OF THE RATIOS OF ROUGH- : SMOOTH-AWNED PLANTS IN F_3' SEGREGATING LINES, COMPARED WITH THE THEORETICAL 3:1

Cross	No.	—	Rough	Smooth	$\frac{\text{Dev.}}{\text{P.E.}}$	Odds
Glabron × Trebi	12	Act. Theor.	536 546.00	192 182.00	1.29	1.63 : 1
Trebi × Glabron	21	Act. Theor.	445 443.25	146 147.75	less than 1	< 1 : 1
Trebi × Glabron	45	Act. Theor.	367 369.75	126 123.25	less than 1	< 1 : 1

From the data just presented it may be concluded that the F_3 behavior of rough-awned F_2 plants supports the proposed two-factor hypothesis for the inheritance of barbed awn.

The behavior of F_3 lines from intermediate-awned F_2 plants is supplied by the data given in Table VIII. A very close fit to the expected is obtained in Cross 21. Certain deviations occur in Crosses 12 and 45 that can possibly be explained by the fact that when only small numbers of smooth-awned plants occurred in segregating lines they were overlooked, and the lines were classified as non-segregating.

TABLE VIII

THE BEHAVIOR OF F_3 LINES FROM INTERMEDIATE-AWNED PLANTS COMPARED WITH THE THEORETICAL RATIO 1:2

Cross	No.	Non-seg.		Seg. 3 intermediate : 1 smooth		χ^2	P
		Act.*	Theor.	Act.*	Theor.		
Glabron × Trebi	12	19	13.66	22	27.32	3.12	0.1–0.05
Trebi × Glabron	21	7	7.00	14	14.00	perfect fit	
Trebi × Glabron	45	13	9.00	14	18.00	2.67	0.1
Total		39	29.66	50	59.32	4.41	0.05–0.02

**Corrected numbers based on F_3 behavior.*

The total numbers of intermediate- and smooth-awned plants in segregating F_3 lines derived from intermediate-awned F_2 plants showed a satisfactory fit to the theoretical in Cross 21 but rather poor fits in Crosses 12 and 45 (Table IX). The deviations noted in the last two crosses were caused by the fact that the segregating lines contained too many intermediate- and insufficient smooth-awned plants.

TABLE IX

SUMMARY OF THE RATIOS OF INTERMEDIATE- TO SMOOTH-AWNED PLANTS IN F_3 SEGREGATING LINES COMPARED WITH THE THEORETICAL 3:1

Cross	No.	—	Intermediate	Smooth	$\frac{\text{Dev.}}{\text{P.E.}}$	Odds
Glabron × Trebi	12	Act. Theor.	685 642.00	171 214.00	5.04	1350.35 : 1
Trebi × Glabron	21	Act. Theor.	404 390.00	116 130.00	2.10	5.38 : 1
Trebi × Glabron	45	Act. Theor.	447 411.75	102 137.25	5.15	1350.35 : 1

It will be recalled that the F_2 material as originally classified included a near-rough class and two intermediate classes designated as smooth-base and near-smooth. As determined by F_3 behavior the plants classed as smooth-base were found to breed true for this condition, whereas those classed as near-smooth segregated into smooth-base, near-smooth and smooth types. On the basis of the two-factor hypothesis proposed for barbing of awn, the true breeding intermediate (represented by the smooth-base type) possesses the genotypic constitution *rrSS* and the segregating intermediate (represented by the near-smooth type) the genotypic constitution *rrSs*. Hence, it is apparent that the hypostatic factor *S* in the homozygous condition causes a more pronounced type of barbing than it does in the heterozygous condition.

The existence of these two intermediate types caused considerable confusion in determining the ratios within lines segregating for intermediate and smooth. Owing to environmental factors, no clear-cut line of demarcation existed between the smooth-base and near-smooth classes or between the near-smooth and smooth classes. These conditions possibly explain the deviations from the theoretical noted in Crosses 12 and 45 in regard to the numbers of intermediate and smooth-awned plants in segregating lines (Table IX).

Plants classified as near-rough in the original F_2 classification behaved either as rough or smooth-base types. Fluctuations caused by environmental factors are probably responsible for this class.

Since it has been shown that the near-rough class was based on environmental rather than on genetic differences, and since smooth-base and near-smooth classes proved to be simply the expression of different genotypes of the intermediate condition expected in the F_2 from a two-factor hypothesis based on epistasis, the grouping adopted in the F_2 appears to be correct.

The F_3 data presented support reasonably well the original hypothesis that two factors explained on the basis of epistasis are involved in barbing of awn. The results are, therefore, in agreement with those reported by Griffee (12) and Robertson, Deming and Koonce (33).

It is interesting to note that the results of this study are not in agreement with those of David (7), who previously worked with the same cross that is discussed in this paper. He reported that two-thirds of the progeny from smooth-awned plants segregated in ratios approximating 3 smooth : 1 rough. No such inverse ratio occurred in the present work. It is possible that these two investigations have been carried out with different strains of Trebi. David describes Trebi as being "six-rowed, white, high yielding, rough-awned". It is not clear in what sense he is using the term "white". If he is referring to the aleurone color, then he is definitely working with a strain of Trebi different from that employed in the present study. The aleurone color of the kernels of the Trebi parent used in this investigation was definitely bluish-gray. This description of Trebi is in accord with that given by Harlan *et al.* (15).

Earliness of Heading

LITERATURE REVIEW

Harlan and Martini (18) in a study of earliness in F_1 barley hybrids found that the hybrids headed quite uniformly as compared with their respective parents. In average date of emergence of awn, the hybrids tended to be intermediate between the parents. The earliness of many hybrid combinations of late varieties indicated that they contained many factors for earliness.

As a result of studying the F_3 lines of a cross between Guy Mayle × Canadian Thorpe, Neatby (30) concluded that growth period was governed by three main factors. He arrived at his conclusions by estimating the number of lines containing plants of winter habit and by comparing the standard deviations of the individual lines with the average of that of the parents. When these three factors are in a homozygous recessive condition winter habit results. Canadian Thorpe was thought to carry two factors and Guy Mayle one.

Griffee (12) found that earliness was governed by a single factor difference with early heading dominant, in a cross between Svanhals and Lion. A distribution table for date of heading of F_3 lines showed them to be of three kinds, *viz.*, early, segregating, and late. These three types appeared in an approximate 1:2:1 ratio.

David (7) studied the inheritance of earliness in the crosses Glabron × Trebi and Velvet × Trebi. The F_1 from each cross was earlier than either parent. When the F_2 plants were graphed on the basis of the number of days from planting to flowering, a ratio of nine early to seven late was obtained. A study of the F_3 lines on the basis of mean number of days from planting to flowering substantiated the view that two important complementary factors were involved in the inheritance of earliness.

METHODS AND EXPERIMENTAL RESULTS

The F_3 lines from two F_2 populations of reciprocal crosses between Glabron and Trebi were used in a study of earliness of heading. This character was not studied in the F_2 as it was thought that a more reliable expression of the time of heading could be obtained from studying the means of F_3 lines rather than the individual F_2 plant. A physiological character such as time of heading is so influenced by environmental factors that data pertaining to individual plants are of no great significance.

The number of plants heading out in each F_3 line was recorded in 2–3 day intervals during the heading period. The emergence of awn, combined with the splitting of the sheath of the first spike, was taken as the criterion of heading. The distribution for date of heading of the plants in each F_3 line was obtained, and from this the mean of each line for the number of days from emergence to heading was calculated.

The expression of this character was affected greatly by environmental conditions. From the behavior of the plants in parental rows, it was evident that considerable variation existed not only within individual rows but also among rows located in different areas of the field. Cool, rainy weather at heading time prolonged heading in the parental rows and in the later hybrid lines of both crosses. This condition made it impracticable to use the distribution of the parental varieties as a measure of homozygosity for the hybrid lines.

The distribution set out in two-day intervals for the mean number of days from emergence to heading, together with the coefficient of variability of parental rows and hybrid lines of Crosses 12 and 21, is given in Table X. Transgressive segregation for both earliness and lateness is clearly indicated, with both of the parental varieties occupying a central position on the distribution surface. Evidently factors for earliness were contributed by both Trebi and Glabron. The distribution of the hybrid lines shows a marked massing in the early classes which would suggest the partial dominance of early heading over late heading. The complex segregation noted in this study of earliness of heading can best be explained by a polymeric factor hypothesis.

Height of Plant

LITERATURE REVIEW

Vestergaard, cited by David (7), reported a segregation of 14 dwarfs to 81 normal plants in the F_2 of a cross between Binder and a dwarf-like variant. Miyake and Imai (28) crossed tall slim plants with short stout ones. The F_1 were tall and slim, whereas the F_2 segregated into a ratio of 3 tall to 1 short. Miyayawa (29) found that dwarfness tended to be dominant in a cross that he studied. A segregation of one sterile dwarf, to two sterile dwarfs, to one normal plant was obtained in the F_2. Harlan and Pope (16), on the other hand, found that a dwarf form behaved as a simple recessive to the normal barley.

Neatby (30) obtained height data on 228 F_3 lines of a cross between Guy Mayle and Canadian Thorpe. No clear indication of the number of factors involved was obtained. By comparing the standard deviations of the F_3 lines with those of the parents, Neatby concluded that at least four factors seemed to be operative.

TABLE X

DISTRIBUTION OF F_3 LINES AND PARENTAL ROWS ACCORDING TO THE MEAN NUMBER OF DAYS FROM EMERGENCE TO HEADING IN RECIPROCAL CROSSES BETWEEN TREBI AND GLABRON

Parent or cross	Classes for number of days													Mean	Coefficient of variability
	48.5	50.5	52.5	54.5	56.5	58.5	60.5	62.5	64.5	66.5	68.5	70.5	72.5		
Trebi	—	—	—	—	2	4	1	1	—	—	—	—	—	58.80	3.37 ± 0.57
Glabron	—	—	—	—	—	1	3	2	2	—	—	—	—	61.75	3.44 ± 0.58
Cross 12, Glabron × Trebi	2	2	13	19	42	27	16	18	10	6	2	3	1	58.65	7.59 ± 0.29
Trebi	—	—	—	—	3	2	—	—	—	—	—	—	—	57.30	1.91 ± 0.41
Glabron	—	—	—	—	2	3	—	—	—	—	—	—	—	57.70	1.90 ± 0.41
Cross 21, Trebi × Glabron	—	6	16	17	20	14	14	6	5	4	2	—	—	57.17	7.61 ± 0.36

David (7) studied plant height in all three generations of the crosses Glabron × Trebi and Velvet × Trebi. The F_1 plants in each cross were taller than the short parent and closely approached the height of the taller. The plants of the F_2 were much more variable than those of the F_1, and parental forms were recovered in both the F_2 and F_3 generations.

METHODS AND EXPERIMENTAL RESULTS

The two reciprocal crosses used for the study of earliness also served as a basis for a study of plant height. The criterion of height was taken as the distance from the base of the culm to the tip of the spike, excluding awns, of the longest tiller. The plants were pulled individually and measured in inches. The variability noted in regard to earliness of heading was even more striking in the case of height of plant. This was again particularly evident in Cross 21.

The distribution of the mean heights of the progeny and parent rows of Crosses 12 and 21, together with their coefficients of variability, are given in Table XI.

TABLE XI

DISTRIBUTION OF HYBRID LINES AND PARENT ROWS FOR MEAN HEIGHT IN INCHES, IN RECIPROCAL CROSSES BETWEEN GLABRON AND TREBI

Parent or cross	Mean height in inches										Mean	C.V.
	27.5	29.5	31.5	33.5	35.5	37.5	39.5	41.5	43.5	45.5		
Trebi	—	—	1	5	1	1	—	—	—	—	34.00	4.88 ± 0.82
Glabron	—	—	—	—	—	—	2	1	4	1	42.50	4.71 ± 0.79
Glabron × Trebi 12	—	1	5	25	47	38	22	19	3	—	36.91	7.74 ± 0.29
Trebi	2	2	—	1	—	—	—	—	—	—	29.40	7.00 ± 1.49
Glabron	—	—	—	—	1	2	1	1	—	—	38.20	5.59 ± 1.19
Trebi × Glabron 21	3	4	27	18	30	15	6	1	—	—	34.28	8.42 ± 0.39

The parent varieties differ quite strikingly in height. Trebi averages approximately eight inches shorter than Glabron. The coefficient of variability of the F_3 lines of Cross 12 as compared with that of the parents, shows the progeny to be considerably more variable in plant height. These differences in variability are not so clear in Cross 21, since, owing to environmental factors, the parental varieties show high variability. However, in view of the fact that the parental varieties occupy the extremes of the distribution surface, while the distribution of the progeny approaches that of the normal curve, it can be concluded that a segregation of genetic factors for height has occurred. Cumulative factors appear to be involved.

Resistance to Loose Smut

Plant disease surveys have shown the loose smut disease of barley to be widespread in Canada (5, 6, 27). However, owing to low percentage infections, the estimated total loss from this disease is considered to be much less than that occurring from the covered smut disease. Loose smut is quite common in western Canada. In 1927, the aggregate loss from loose smut occurring in Alberta has been reported as being considerable (27, 36). In Manitoba in 1930 loose smut was found in 22 fields out of 26 examined. However, the estimated damage was considered to be only one-half of one per cent (6).

It is a very significant fact that the existing smooth-awned barley varieties are susceptible to this disease. It is possible that, when smooth-awned varieties are grown to a greater extent in western Canada than they are at present, the plant disease surveys will show a correspondingly higher loss from loose smut. It is also important to note that the disease is not controlled by the simpler methods of seed treatment.

Until comparatively recently it was believed by the majority of investigators that only two distinct types of barley smut existed: covered smut (*Ustilago hordei*) causing seedling infection, and loose smut (*Ustilago nuda*) capable of causing infection only through the young embryo. Seed surface disinfectants have been advocated to control covered smut, while the hot water treatment was thought to be the only means of controlling loose smut. However, about 1923, experiments conducted with seed disinfectants showed that loose smut could be controlled in some varieties by seed treatment with formaldehyde and certain mercury compounds (45, 46). These results suggested that infection with loose smut may take place through seed-borne spores. This possibility was investigated by Tisdale and Tapke (47) who found that dehulled seed of a number of varieties, when inoculated with spores of *U. nuda* obtained from the variety Tennessee Winter, produced plants with a high percentage of loose smut.

Further studies on this type of loose smut infection by Tisdale and Griffiths (48) showed that *U. nuda* was composed of a number of variants (physiologic forms). These were found to vary not only in pathogenicity but also in the manner in which they affected the germination and development of the host plant. These investigators postulate that there are in existence strains of *U. nuda* capable only of flower infection while others appear to infect primarily through the seed. It is significant to note in this connection that Rodenhizer (34) found greater cultural differences between different forms of *U. nuda* than between *U. nuda* and *U. tritici*. More definite information in regard to possible smut strains was supplied by Tapke (43) in 1932, who found that loose smut could be caused by either of two fungi: *Ustilago nuda* (Jensen) K & S., and a new species which he has named *Ustilago nigra*. The former species was unable to cause seedling infection and could not be controlled by seed surface disinfectants. The latter species, on the other hand, caused seedling infection, and was readily controlled by Ceresan dust or liquid formaldehyde treatment of flower-inoculated seed. The spores of *U. nuda* also appeared to be considerably shorter lived than those of *U. nigra*. Tapke intimates that the divergent result obtained from seed treatment for the control of loose smut was due to a failure to recognize these two types of smut.

The existence of a smut type intermediate between *U. hordei* and *U. nuda* was discovered as early as 1894 by Biedenkopf (3). This type which he named *Ustilago medians* produced smutted spikes similar to those produced by *U. nuda*, but behaved as *U. hordei* in its manner of spore germination.

More recently Vanderwalle (49) has described a late developing smut type which he considers to be intermediate between *U. hordei* and *U. nuda*. This type was incapable of producing seedling infection.

Ruttle (35) has described five types of smut intermediate between *U. nuda* and *U. hordei*. One of these (Type 4) was found to correspond quite closely to the *U. medians* Biedenkopf and *U. nigra* Tapke. Types 2 and 3 were intermediate between Type 4 and *U. hordei*, while Types 5 and 6 were intermediate between Type 4 and *U. nuda*. Type 3 corresponded somewhat in morphological features with the late developing type of Vanderwalle. Ruttle points out that a large proportion of the smut collected in New York state was of this intermediate nature, and, while seemingly true loose smuts, were actually seedling infection and readily controlled by seed disinfectants. She states, furthermore, that spore germination is the only certain way of distinguishing between the intermediate seedling infecting and the true loose smuts.

The relation of environment to the development of the loose smut in barley appears to have received relatively little study. Seiffert, cited by Leukel (25), and Taylor and Zehner (44) found that shallow-sown seed produced less infected plants than seed sown deeply. Kirby (24) believed that soil temperatures higher than the optimum for the development of the barley seedling favored the development of loose smut. Tapke (42) studied the influence of humidity on floral infection of wheat and barley with loose smut. He concluded that relatively low humidity during the period of flowering tended to inhibit infection with loose smut in both wheat and barley. Leukel (25) found that a very wet soil (90% saturation) inhibited loose smut development somewhat, and favored its control by seed-surface dust fungicides. A very dry soil had the reverse effect. A relatively high soil temperature before emergence appeared to favor loose smut development.

In the foregoing paragraphs an attempt has been made to present a brief statement of the status, at least in America, of the loose smut disease of barley. There are in existence a number of barley smut types intermediate between *U. hordei* and *U. nuda*, both in morphological features and in their mode of infection. A number of these, while producing disease symptoms similar to those produced by *U. nuda*, are primarily seedling infecting and are amenable to control by seed disinfectants. The latter have been found to control loose smut only in certain varieties indicating that some varieties may be susceptible to the flower-infecting types of smut, while others may be susceptible primarily to the seedling-infecting types.

It is evident from the work reviewed above that the problem of loose smut in barley is a complicated one and in need of further investigation. In this connection Ruttle (35) states "further study is needed on the barley smut types, on their field occurrence, their mode of infection, their control, their biologic forms, their host reactions, and primarily on their relationship to *U. nuda* and *U. hordei* and to one another".

There is a great need in western Canada for just such studies. Undoubtedly intermediate types of smut occur in this area, but as yet no one has separated them. It is obvious that no progress can be made in devising control measures for the loose smut disease, or in the breeding of resistant varieties, until the prevailing type and its method of infection are ascertained. Since in all likelihood the varieties to be developed in the future will be smooth-awned types, it is imperative that information regarding the loose smut disease be obtained at an early date so that the desirable combination of smooth awn and resistance to the loose smut disease can be incorporated within these varieties.

At the University of Alberta, in 1931, at attempt was made to test the reaction of the F_3 progeny of crosses between Trebi and Glabron to *U. nuda.* The method of seed inoculation as described by Tisdale and Tapke (47) was used. The inoculum used was collected from a commercial field of smooth-awned barley in the vicinity of Edmonton, Alberta. Although the inoculum showed a comparatively high percentage of germination just prior to inoculation, the resulting plants from inoculated kernels were entirely free from loose smut. It was about this time that Tapke (43) described the new species of loose smut, *U. nigra*, capable of causing seedling infection as contrasted with the true loose smut, *U. nuda*, capable only of floral infection. Examination of the inoculum used in the study reported revealed spores typical of *U. nuda.*

The results of this study serve to emphasize the need for detailed information concerning the smut fungi prevalent in western Canada, before progress can be made in breeding new varieties resistant to these diseases.

Resistance to Stripe

LITERATURE REVIEW

Plant disease surveys have shown that, from the standpoint of distribution and destructiveness, the stripe disease is generally less important economically than the covered smut disease (5, 6). However, periodically, owing possibly to environmental conditions, this disease becomes quite destructive. Thus in the year 1920 stripe was considered the worst disease of the barley crop in the prairie provinces (8). Sanford (36) states as a result of a disease survey of Alberta conducted in 1927 that stripe was common and at times severe. In the year 1928 barley stripe was reported as being quite prevalent in Manitoba, especially in late low-lying fields where 60% of the plants were infected (27). Twenty-five out of 108 fields examined in Alberta in 1930 showed striped plants. Four of these were classed as heavily infected with one field showing 20–30% infection (6). It is worthy of note that in the plant disease survey reports, reference has been repeatedly made to relatively high infections of stripe observed in varietal plots at experimental stations when little was found in surrounding commercial fields. This condition may be partially due to the fact that farmers generally sow their barley at a considerably later date than is practised at experimental stations, and therefore at a time when soil temperatures are high and inhibitory to stripe development.

Methods of Infection

Until recently the stripe fungus was thought to infect plants at flowering time and to cause a systemic infection similar to that of loose smut of wheat and barley in plants from infected seed. Several studies regarding the mode of infection of this fungus have modified the general views in this regard. Ravn (32) believed that *H. gramineum*, like certain smut fungi, inhabits the growing point of the host plant and thence spreads to each young part of the plant during the formation of that part. He found that the bulk of the infection occurred at flowering time and that low percentages of infection occurred when germinated seedlings were grown in the presence of mycelium. Vogt (51) as a result of histological studies showed the mycelium inhabited the space between the glumes and the pericarp and is never found in the embryo or in the endosperm. Infection was found to take place through the coleoptile rather than the embryo. Smith (40) came to similar conclusions in that he found infection to occur while the shoot is still under the adherent glumes or during its emergence. The inoculum was found to comprise: (a) conidia lodging at the awn end of the grain, (b) mycelium penetrating from the glumes, and (c) perithecia formed inside or outside the glumes. Johnson (22) showed that it was possible to obtain artificial infection of barley simply by inoculating the germinating kernels with spores or mycelia. Dehulling of the kernel increased the infection considerably.

In a later paper, Smith (41) showed definitely how infection of barley seedlings takes place. Mycelium is considered to be the main source of inoculum and is capable of living for at least two years in the chaff and pericarp. On the germination of the kernel the hyphae infect the primary sheaths, the coleorhiza (leading to root decay) and the coleoptile (causing leaf disease). From the coleoptile each successive leaf as formed is infected—the lesions on the newly infected leaf paralleling those on the older leaves enclosing it. The uppermost infected leaf may cause external infection of the young spike. Hence it can be seen that the mode of infection is contrary to that of smut infection. That is to say, the leaves are infected first and the growing point later, whereas, in the smuts the growing point is first infected and the other parts attacked as they are formed.

Temperature Relations

Several investigators have demonstrated that low soil temperatures favor infection of barley seedlings with *H. gramineum*.

Ravn (32) found that early planting produced greater infection. Johnson (22) showed, by the use of controlled experiments, that low soil temperatures are favorable to increased infections. He found the optimum temperatures for infection to be 10–12° C., while little occurred at soil temperatures higher than 20° C. Smith (41) points out that low soil temperatures greatly slow down germination processes, thus increasing the likelihood of infection while the shoot is still confined within the chaff. Isenbeck (21) found that soil temperatures varying from 0–8° C. with 25% soil moisture were most favorable for stripe infection. Leukel, Dickson and Johnson (26) stated that temperatures of 15° C. or less during the period of emergence favored stripe infection while the disease was inhibited by temperatures above 20° C. In a recent paper concerning the relation of temperature to the stripe diseases, Shands (37) has stated that "in analyzing the effect of temperature on the development of stripe, such reactions as the effect of temperature on the host, on the parasite and on the combination of the two in the production of disease must be studied." He points out that barley is a relatively low-temperature plant making best development at constant low soil temperatures of approximately 12–16° C. Highest stripe percentages also occurred at these constant low temperatures when the inoculated kernels were incubated at the different temperatures until they had reached approximately the same stage of development. However, when the kernels were incubated for equal periods of four days at each temperature, the greatest percentage of stripe developed at about 20° C. Change of temperature from low to high stimulated disease appearance, while the reverse change retarded disease expression.

Physiologic Specialization

Physiologic specialization in this fungus was first demonstrated by Johnson (22). A single spore isolation obtained from Edmonton, Alberta, failed to recover when transferred to room temperatures after being kept at 32°–33° C. for 10–12 days, while several other cultures tested in a similar manner recovered and grew normally. The Edmonton form also grew better at 5–6° C. than did the other cultures under test.

Christensen and Graham (4) distinguished between more than 125 races of *H. gramineum* in culture from collections obtained from 12 states of the United States and from Canada and Germany. The type of medium as well as bacterial association were found to affect races differently. Great variability in the stability of these races was noted. At least 20 races could be distinguished by their relative pathogenicity on 16 varieties of barley. Isenbeck (21) demonstrated the presence of at least three physiological forms of *H. gramineum* based on cultural characteristics and differences in pathogenicity. An American form studied proved to be less virulent than the German forms studied.

Inoculation Methods

Ravn (32) found the greater percentage of successful infections to result from floral inoculation and that low percentage of infection resulted from germinating seedlings in the presence of the mycelium.

Johnson (22) was probably the first investigator in America to demonstrate that it was possible to obtain stripe infection by seed or seedling inoculation. The period of greatest susceptibility in dehulled kernels was found to be that just subsequent to the emergence of the coleoptile.

Genau (10) found the optimum time to effect floral inoculation with conidial suspension was the day following opening, provided the temperature approximated 25° C. Young seedlings 1–2 cm. in height were found to be readily infected by means of a conidial suspension in sufficiently warm and humid atmospheres. In one experiment the plants were successfully inoculated with the ascospores of the fungus.

Fuchs (9) has devised a method of artificial inoculation consisting of the injection of a conidial suspension of the fungus into the ripe grain after soaking the latter for an hour or two in water so as to loosen the glumes from the grain and permit of inserting the needle between them. For large quantities of inoculum a modified method was devised. The barley grains are placed in a vacuum for 20 minutes in a conidial suspension in such a way that the air between the glumes and seed is removed. When the pressure is restored the conidial suspension penetrates with great impetus between the glume and the caryopsis. To stimulate abundant mycelial production, the inoculated grains are placed in an incubator at temperatures of 25–28° C. and at an atmospheric humidity of 90–100%. It is claimed that this method gave results in complete agreement with the floral method used by Genau (10).

Isenbeck (21) made a thorough study of inoculation technique in regard to the stripe disease. He investigated five infection methods as follows:

1. Floral inoculation using a wet conidial suspension.
2. Floral infection using dry conidia.
3. Seedling inoculation.
4. Grain inoculation using spores.
5. Grain inoculation using mycelia.

In both types of floral inoculations the inoculum was introduced between the flowering glumes and palea of each individual flower. The dry method gave consistently higher stripe infection than the wet method. Generally, floral inoculations gave uncertain results in that they were readily influenced by environmental factors.

Seedling inoculation, after being fully investigated, was discontinued owing to the fact that the resulting disease symptoms were distinctly abnormal.

Dehulled kernels were used in both methods of grain inoculations. The spore method consisted of rolling the kernels while damp in spore dust, while in the mycelium method the kernels were mixed with small pieces of agar containing mycelium and later laid out in Petri dishes to germinate. The grain inoculations resulted in high infections and generally gave much more certain results than floral inoculations. Only 4–5 weeks were necessary for testing when seed inoculation was practised, while over a year was necessary in the case of floral inoculations.

Shands (37) obtained relatively high infections of stripe when plants were enclosed in glassine bags just prior to heading and sprayed with a conidial suspension of the fungus. Seed inoculations of dehulled kernels, effected by placing them between layers of potato dextrose agar on which the fungus was growing and incubating overnight before planting, also gave good infections.

Genetic Studies

Isenbeck (21) studied the reaction to *H. gramineum* of the progeny of eight crosses involving different combinations of immunity, resistance and susceptibility in the parents. Flowers of F_2 plants were inoculated thus giving ratios for the F_3. The F_4 was also studied in some cases. All the crosses were tested by the method of wet infection, two by dry infection and one F_3 was also tested in the greenhouse by the infection of the grain. Generally immunity was found to be dominant; crosses between two immune varieties gave no susceptibles, whereas crosses between two resistant types resulted in some susceptible types. Transgression also occurred when two susceptibles were crossed. The results can only be explained by assuming a large number of factors.

Shands *et al.* (38) studied stripe reaction in the progeny of a number of crosses between Oderbrucker, a white rough-awned variety and Leiorrhynchum, a small black smooth-awned variety. A wide range of variation from susceptible to highly resistant selections was found. Back crossing to the Oderbrucker parent resulted in increased susceptibility to the disease. The resistance of smooth-awned selections under field conditions was confirmed by results obtained from artificial inoculations. Stripe development was found to be readily influenced by environmental factors. Their results, while showing the heritable nature of stripe reaction, fail to give any indication of the number of factors involved.

METHODS AND EXPERIMENTAL RESULTS

A method of floral inoculation, using a wet conidial suspension, was utilized in the present inheritance studies. The inoculum consisted, in the main, of diseased leaves of badly infected plants from a plot of Canadian Thorpe; this was supplemented from time to time by a composite sample of inoculum from the varietal plots. The infected leaves were placed in an incubating chamber overnight to stimulate spore production. The following day the leaves were rubbed together under water until they were disintegrated. The spore suspension was then strained through three layers of cheesecloth to remove all suspended vegetative matter. Two spikes from approximately 500 F_1 plants of crosses involving Trebi and Glabron, as well as two spikes of 100 parental plants, were selected at random for inoculation. This consisted of dipping the spikes into the solution and immediately enclosing them in glassine bags. The spikes were given additional sprays at four to five day intervals by means of an atomizer, the nozzle of the latter being inserted through slits cut in the glassine bags. The weak straw of the barley plant caused considerable difficulty in that it was necessary to support the majority of the bagged spikes. Even with this precaution a number of spikes were broken over by the wind and rain.

The kernels from the inoculated spikes were sown as early in the spring of 1932 as was deemed practicable with soil temperatures ranging from 10–13° C. The infected plants of both hybrid and parental lines showed the characteristic striping of the disease just prior to heading. Little or no partial infection was evident, all infected plants being ruined economically (See Fig. 2). The infection classes in percentage for the parental rows and F_3 hybrid lines are given in Table XII.

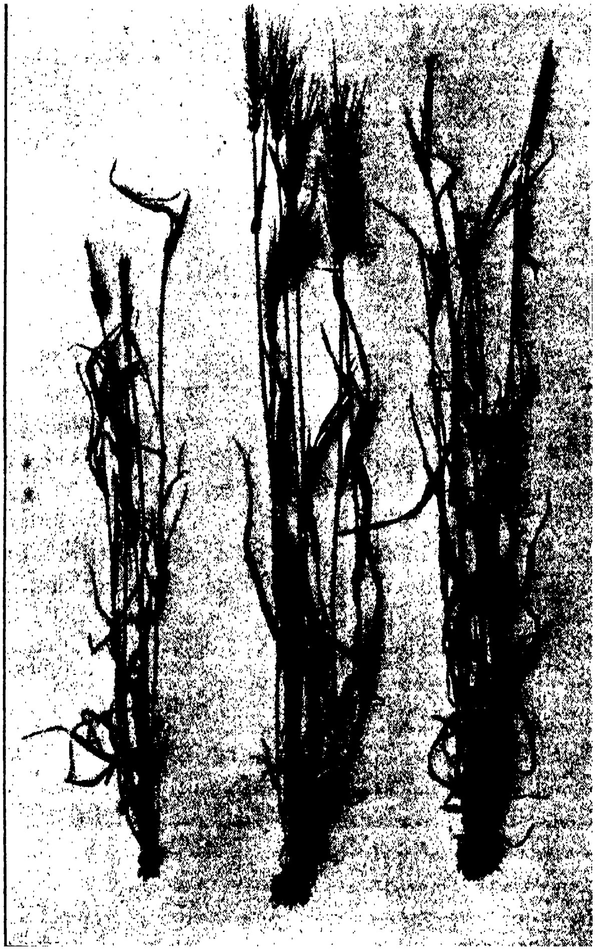

FIG. 2 *Barley plants infected with stripe, Helminthosporium gramineum. Normal plant in the centre. (Photograph taken when normal plants were approaching maturity).*

TABLE XII

REACTION OF F_3 LINES AND PARENTAL VARIETIES TO *H. gramineum* IN RECIPROCAL CROSSES OF GLABRON AND TREBI

Cross or parent	Infection classes in percentage								
	0	3	8	13	18	23	28	33	58
Trebi	39	8							
Glabron		8	14	12	7	5	1		
126 lines, Glabron × Trebi	36	40	31	13	3	—	1	1	1
336 lines, Trebi × Glabron	137	93	48	20	11	5	1	—	—
Total, 462 lines	173	133	79	33	14	5	2	1	1

The Trebi parent appears to be highly resistant to the form of the pathogen (*H. gramineum*) used, while Glabron shows infection varying from 3% to 28%, with the greatest number of lines showing from 8% to 13% infection. The wide range of infection exhibited by Glabron would indicate certain limitations in the inoculation methods employed. There is no evidence of any transgression toward greater susceptibility in the hybrids except in one line, which gave 58% infection and to which no particular significance can be attached. Isenbeck (21) found that the number of resistant individuals in the progeny increased with the degree of resistance of the two parents. The present results bear out this conclusion. Keeping the parental types in view, it is extremely difficult to postulate any definite mode of inheritance. It is important to point out, at this time, that the plants selected in the F_2 for inoculation were really a random distribution of the early plants, since there had been a tendency to select such plants for inoculation purposes. This may have possibly influenced the results obtained.

The results of this study serve to emphasize the need, when studying the reaction of a hybrid population to infection with *H. gramineum*, of inoculation methods that will give relatively certain results. The wide range of infection percentages exhibited by the Glabron parent indicates that, in the method of inoculation employed, the percentages of kernels successfully infected per inoculated spike varied considerably. Isenbeck (21), after having made a careful study of a number of inoculation methods stated . . . . "It is evident that the method of flower infection, even if thoroughly worked out, will never be as reliable as a method of grain infection, the whole of which can be carried out in the greenhouse under strictly controlled uniform conditions. Flower infection is dependent upon so many factors which cannot be controlled or even known, that a high degree of certainty of infection is hardly possible, especially in the very delicate work with *Helminthosporium*."

The same worker concludes that for varietal tests where only a comparative stripe reaction is desired, inoculation by the dry spore dust (field infection) should be practised, as this method most nearly approaches that occurring under natural conditions. On the other hand, when absolute infection percentages are desired as in genetic studies, greenhouse infections, involving kernel inoculation by either spore dust or mycelia, should be used.

Correlation Studies

In an endeavor to obtain further information regarding the nature of the inheritance of stripe reactions, simple correlations were calculated between stripe infection percentages and both mean height of plant and mean number of days from emergence to heading; also a test of independence or association (11) was made between barbing of awn and stripe reaction. Mean height of plant was correlated with mean number of days from emergence to heading. The correlation coefficient values obtained in this study are summarized in Table XIII.

TABLE XIII

CORRELATION VALUES OBTAINED BETWEEN CHARACTERS OF F_3 HYBRID LINES OF RECIPROCAL CROSSES BETWEEN GLABRON AND TREBI

Characters correlated	Cross number	Correlation coefficient	P
Mean height and per cent stripe infection	12 and 21	0.388	0.05
Mean number of days to heading and per cent stripe infection	12 and 21	0.124	greater than 0.50
Mean height of plant and mean number of days to heading	12	0.728	less than 0.01
	21	0.737	less than 0.01

High significant correlations were demonstrated between mean height of plant and mean number of days from emergence to heading. Although this association may be partially explained on a purely physiological basis, its high value indicates that certain of the factors responsible for earliness of heading also determine plant height. No significant correlation was shown between mean number of days to heading and percentage stripe infection.

The value $r = 0.388$ obtained between mean height of plant and per cent stripe infection is statistically significant in the light of its P value, .05. As tallness of plant and susceptibility to the stripe disease entered the cross together, this value may indicate a slight genetic linkage.

A test of independence or association calculated between barbing of awns and per cent stripe infection gave a P value of 0.27 which is indicative of a lack of association between these characters.

A number of individual plant selections were made, based on stripe resistance, smoothness of awns, earliness of maturity and strength of straw. These are now being compared with the standard varieties in the regular varietal test plots.

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THE EFFECT OF POTASSIUM SUPPLY ON THE COMPOSITION AND QUALITY OF WHEAT. II[1]

By A. G. McCalla[2] and E. K. Woodford[3]

Abstract

Limiting the supply of potassium to wheat plants resulted in a decreased nitrogen content and a markedly increased calcium and magnesium content of the dry matter. It retarded growth, reduced the total amount of individual nutrients absorbed per plant, and resulted in an increase in the proportion of grain to total yield. It had an adverse effect on the quality of the grain as determined by gluten and baking tests.

Neither sodium nor calcium was effective in replacing potassium, but sodium was the better in this respect.

Introduction

In an earlier paper (9), it was pointed out that the potassium nutrition of the wheat plant markedly influenced the quality of the grain produced, although it apparently affected the nitrogen and mineral content of the kernels but little. The importance of these findings led the writers to repeat the earlier work and, in addition, to study the distribution of nitrogen and minerals in the developing plants.

Representative literature relating to this problem was reviewed in the earlier paper. Since it was written, Hibbard and Grigsby (5) have published the results of a study of pea plants grown in limited potassium and calcium solutions. It was concluded that limiting potassium had little if any effect on the total nitrogen, but that it reduced the ash and increased the calcium content.

Experimental

Production of Material

The material used in this study was Marquis wheat grown in nutrient solutions during the spring and summer of 1934. Five cultures were produced in tanks measuring 30 × 60 × 8 in. The plants, 512 in each tank, were grown on a seedbed of sawdust spread over burlap in the manner described by Gericke (3). The solutions supplied to the cultures are recorded in Table I, and the chemical composition of the various solutions in Table II.

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TABLE I

SOLUTIONS SUPPLIED TO THE VARIOUS CULTURES

Culture No.	First month	Remainder of growth period	Abbreviated description
1	Complete	Complete	Complete
2	Complete	K-free with Na	Complete : K-free, Na
3	Complete	K-free with Ca	Complete : K-free, Ca
4	Low K with Na	Low K with Na	Low K, Na
5	Low K with Ca	Low K with Ca	Low K, Ca

TABLE II

CHEMICAL COMPOSITION OF SOLUTIONS EXPRESSED AS CC. OF MOLAR SOLUTION PER LITRE

Solution	KNO_3	$Ca(NO_3)_2$	$NaNO_3$	KH_2PO_4	$Ca(H_2PO_4)_2$	NaH_2PO_4	$MgSO_4$
Complete	5	5	—	1	—	—	2
K-free, Na	—	5	5	—	—	1	2
K-free, Ca	—	7.5	—	—	0.5**	—	2
Low K, Na	—	5	5	0.15*	—	0.85	2
Low K, Ca	—	7.5	—	0.15*	0.42**	—	2

**Added as 0.5 cc. once every 10 days.*

***Added as M/16 $Ca(H_2PO_4)_2$.*

The tanks were drained at the end of one month, and refilled with the solutions listed in Table I. At the time of heading the tanks were again drained and refilled. The treatment after heading was therefore quite different from that of the previous experiment in which tap water was used from this stage until maturity.

Ferric tartrate (0.5%) was added to the solutions at the rate of 1 cc. per litre every third day for the first month and then every second day until vegetative growth ceased.

The first seeding was made on April 17, 1934, but when the plants were 44 days old, an accident in the greenhouse killed a large proportion and the series had to be discarded. A second seeding was made on June 2, and the plants grown to maturity.

Sampling was started 20 days after the seeding of the first series. Fifty plants were gathered at random from each tank and brought to the laboratory where they were immediately weighed and analyzed. Similar but smaller samples were collected from the first series at the end of 31 and 44 days, and of the second series after 31 and 54 days. The first heads appeared at the time of the last collection. The plants remaining in each tank were harvested when mature, threshed, and used in composition and quality determinations.

METHODS OF ANALYSIS

With the exception of the determination of potassium, the methods used in this investigation were the same as those employed in earlier work (8, 9). Potassium was precipitated with sodium cobaltic nitrite and the precipitate titrated with $N/20$ potassium permanganate. The details of the procedure are those described by Hibbard and Stout (6).

Results

COMPOSITION OF PLANTS

It was unfortunate that the first series of plants had to be discarded after 44 days, but the second series was grown under similar cultural conditions. The results of the analysis of the two lots of 31-day-old plants are shown in Table III. The actual numerical values for the two series varied somewhat, but with one exception the order of the cultures was the same for each constitutent determined. Since the differences among cultures, rather than the progressive physiological development of the individuals, are stressed in this paper, the variations between the two series of plants are insignificant when compared with the variations among the different cultures.

TABLE III

COMPOSITION OF 31-DAY-OLD PLANTS FROM THE TWO SERIES

Culture No.	Series No.	T.N., % of D.M.	Ash, % of D.M.	K, % of ash	Ca, % of ash	Mg, % of ash	P_2O_5, % of ash
1	1	5.43	16.7	41.2	4.15	1.84	16.8
1	2	4.30	13.6	38.0	5.22	1.17	11.2
4	1	4.90	10.0	18.7	8.47	4.56	27.3
4	2	3.90	7.4	17.4	11.13	4.99	21.2
5	1	4.76	9.2	21.6	12.41	5.34	28.0
5	2	3.82	7.2	18.1	15.67	4.76	22.0

It seemed preferable therefore to present the results obtained with the developing plants graphically, and to plot the values for the 20-, 31- and 44-day-old plants of the first series with those for the 54-day-old plants of the second series. A break between the points for the 44- and 54-day-old plants has been left in each curve on the graphs, so that the reader will bear in mind that the two series of plants for which these results were obtained were not strictly comparable.

The results dealing with the composition of the developing and mature plants are considered separately because the amount and distribution of any one constituent may be considerably different after vegetative growth ceases than before. This has been demonstrated in earlier work on nitrogen metabolism (8). In deficient cultures, absorption of certain nutrients may continue as long as there is vegetative growth, but may cease when such growth ceases. On the other hand the same nutrients may be absorbed from a complete solution after vegetative growth is complete. The more important of such effects are noted in the following discussion.

The plants grown in the complete : potassium-free, calcium culture did not develop normally for more than 10 days after the transfer to the potassium-free solution. The roots became dark in color and gradually rotted away. These effects had become quite definite by the time the plants were 54 days old, and probably account for some of the apparently anomalous results presented in the following sections. This culture produced only about 8 gm. of grain, which was insufficient for the various tests carried out on the other samples. Normally the production of grain takes a large proportion of the nitrogen and a considerable proportion of the minerals from the vegetative parts. Since this transfer did not take place, the mature plants were abnormal in composition. All results pertaining to this particular culture are therefore omitted from the tables and discussion dealing with the mature plant.

Nitrogen

Developing plants. The results for total and nitrate-free nitrogen as percentages of the dry matter are presented in Fig. 1. The complete culture plants absorbed a higher proportion of total nitrogen than did those grown in

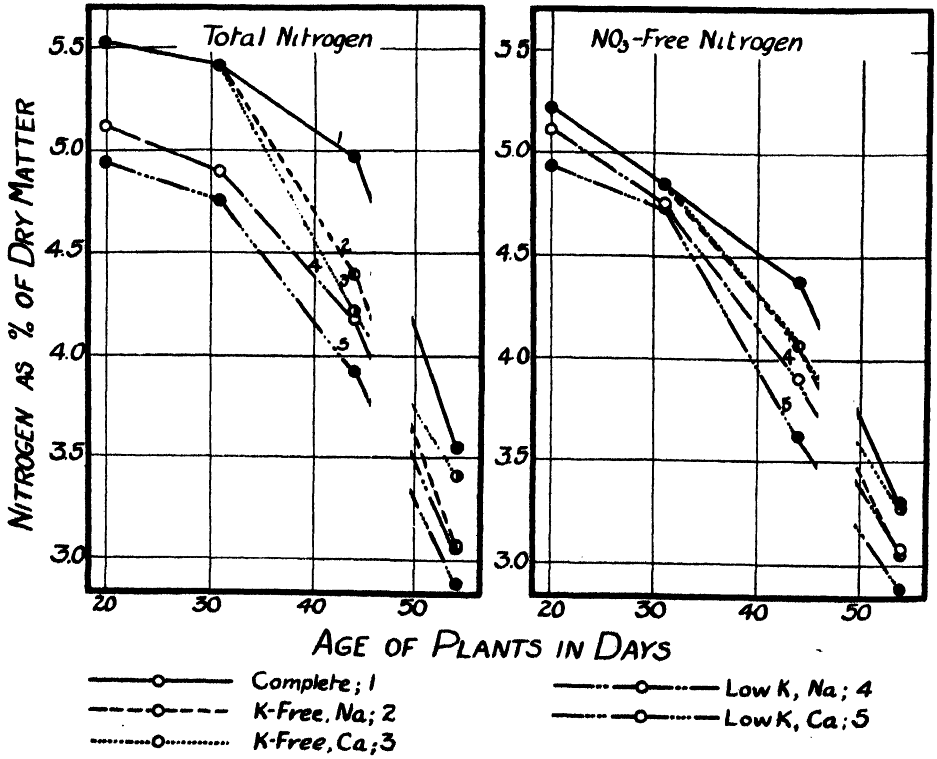

FIG. 1. *Total and nitrate-free nitrogen expressed as percentages of the dry matter in relation to age of plants and potassium nutrition.*

any of the other solutions. The substitution of sodium for potassium resulted in a greater proportionate uptake than the substitution of calcium. This was true both for the plants grown in the complete solution for one month and then transferred to the potassium-free solutions and for those grown in the low potassium solutions at all stages. The only exception is the nitrogen content of the 54-day-old plants grown in potassium-free, calcium solution and this is believed to have been affected by the relatively poor growth of these plants after their transfer to the potassium-free solution.

The nitrate-free nitrogen was also highest in the complete culture plants, but the difference between the content of these plants and that of any one of the others was considerably less than that found for the total nitrogen. This was of course reflected in the nitrate nitrogen content, this form of nitrogen being present in the complete culture plants in more than twice the quantity found in the plants grown in any other solution. The nitrate content of the various collections varied irregularly, but at the time of the 54-day collection there was no nitrate in the plants from Cultures 2, 4 and 5, while the complete culture plants contained over 7% of their total nitrogen in this form.

The results for organic non-protein nitrogen are shown in Fig. 2. The complete culture plants were definitely lower than the others in this form of nitrogen, and the plants grown in high calcium solutions were higher than the comparable plants grown in solutions in which sodium had replaced potassium. This relation appears to be altered at the time of the fourth collection. Even before this, the development of the plants in the two high calcium solutions had been interrupted. The complete : potassium-free, calcium plants did not mature normally, while the low potassium, calcium plants were small and light.

The higher proportion of non-protein nitrogen has been observed by various workers studying plants grown in potassium deficient solutions. That this proportion is higher in plants grown in low potassium solutions at all stages suggests that the course of metabolism is more disturbed by a continuously low supply of potassium, than by complete absence of this element following a normal supply for one month. The decrease in the non-protein nitrogen content of the 54-day-old plants grown in the high calcium solutions probably

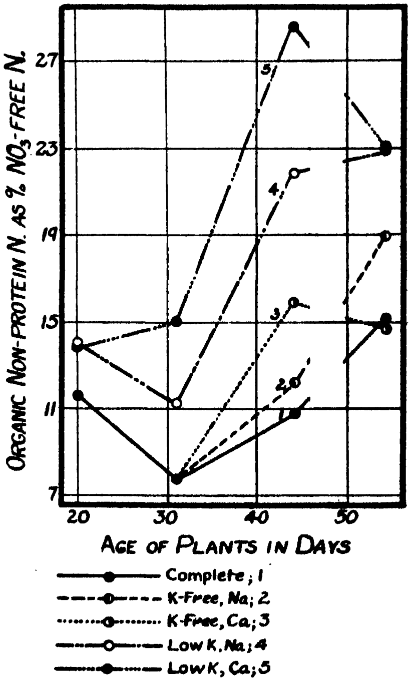

FIG. 2. *Organic non-protein nitrogen as influenced by age of plants and potassium nutrition.*

does not mean that these plants were becoming more normal in their development, but rather that the lack of potassium was curtailing not only growth, but also absorption, with the result that the non-protein nitrogen present in the plants was being utilized in the production of proteins for new growth faster than it was being formed by the reduction of nitrates. It was found in an earlier study (8) that the removal of nitrogen from the nutrient solution at the time the wheat was heading resulted first in a decrease in the percentage of non-protein nitrogen. This decrease was believed to be directly due to the removal of the non-protein nitrogen already present to form proteins in the developing heads. In the present experiment the same explanation is valid, except that it was potassium supply which was limiting nitrogen absorption.

The deficiency of potassium did not appear to curtail the nitrate reduction in any of the plants, but it did curtail absorption. It also reduced the condensation of the non-protein nitrogen compounds to protein. The last two results are in agreement with those obtained from most other experiments of this type (9).

Mature plants. The total amounts of nitrogen absorbed by the plants remaining in the tanks after samples of the developing plants had been collected are given in Table IV. These figures, which are for the same number in each culture, are referred to as the total absorption of the cultures, although it is recognized that at some stage in the development of the plants there may have been a larger content of some individual element. This is certainly true of potassium, which is known to be excreted during the last weeks of the plant's growth.

TABLE IV

NITROGEN AND MINERALS ABSORBED BY PLANTS GROWN TO MATURITY

Culture No.	Description	Total N	NO_3-free N	Ash	K	Ca	Mg	P_2O_5
As gm. per 100 gm. dry matter								
1	Complete	2.63	2.23	12.56	4.60	1.01	0.12	0.90
2	Complete : K-free, Na	2.14	2.05	7.37	0.85	0.85	0.18	1.04
4	Low K, Na	2.06	1.99	8.18	0.93	0.96	0.15	0.84
5	Low K, Ca	2.33	2.25	8.58	1.42	1.45	0.15	0.89
As total gm. per tank								
1	As above	18.15	15.35	86.70	31.78	6.98	0.80	6.22
2	As above	14.37	13.76	49.46	5.71	5.71	1.24	6.96
4	As above	9.93	9.56	39.35	4.47	4.60	0.70	4.02
5	As above	5.89	5.70	21.70	3.59	3.66	0.37	2.25

The complete culture plants absorbed more total nitrogen per 100 gm. of dry matter than did any of the others, but a larger proportion of the absorbed nitrogen remained in the form of nitrates. The low potassium, calcium culture was as high as the complete in nitrate-free nitrogen, although in the earlier stages it was considerably lower. This change may be attributed to its restricted production of vegetative material during the later stages of growth. This question is considered more fully in the final section of the paper. The figures for total nitrogen absorption per tank show quite definitely the superiority of the complete culture and the marked inferiority of the culture in which calcium replaced potassium. Apparently the course of absorption was less impaired by the use of the complete solution for one month followed by a complete absence of potassium than by the maintenance of a low potassium supply at all times, although the amount of potassium per 100 gm. of dry weight was actually the lowest in the plants from the complete : potassium-free sodium culture.

Ash and Ash Constituents

Developing plants. The ash results are shown in Fig. 3. The complete culture plants contained much more ash than the low potassium plants, and the difference was maintained throughout the period studied. The percentage of ash in the plants which were transferred from complete to potassium-free solutions at the end of the first month decreased rapidly, and 23 days after the transfer had reached a value almost as low as that of plants which had a limited potassium supply at all stages. The rapid fall in the ash percentage shows the importance of the part played by potassium in the normal absorption by the plant, which is also demonstrated by the marked similarity between the ash and potassium (Fig. 4) curves. The calculation of the correlation between these two constituents gave a coefficient of $+.96 \pm 0.02$, and although this figure is based on only 16 samples, its significance can hardly be questioned.

The results of mineral analyses expressed as percentages of the dry matter are presented in Fig. 4, and as percentages of the ash in Fig. 5.

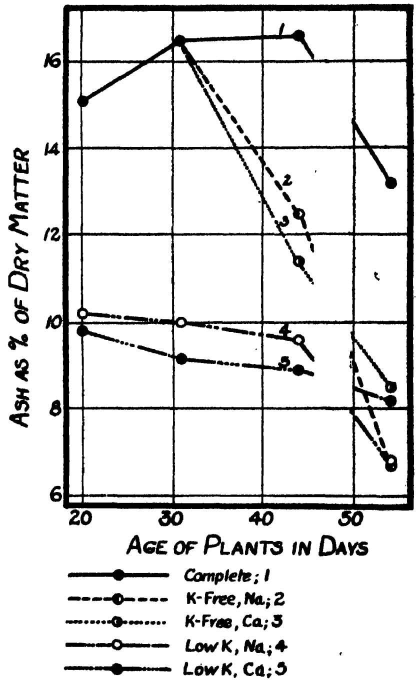

FIG. 3. *Ash content of the dry matter in relation to age of plants and potassium nutrition.*

The complete culture and low potassium plants maintained a fairly constant potassium level during the time the plants were studied, although the former contained approximately twice as much potassium as the latter. In the plants transferred to a potassium-free solution at the age of one month, the potassium content expressed either as a percentage of the dry matter or as a percentage of the ash, decreased rapidly after the transfer.

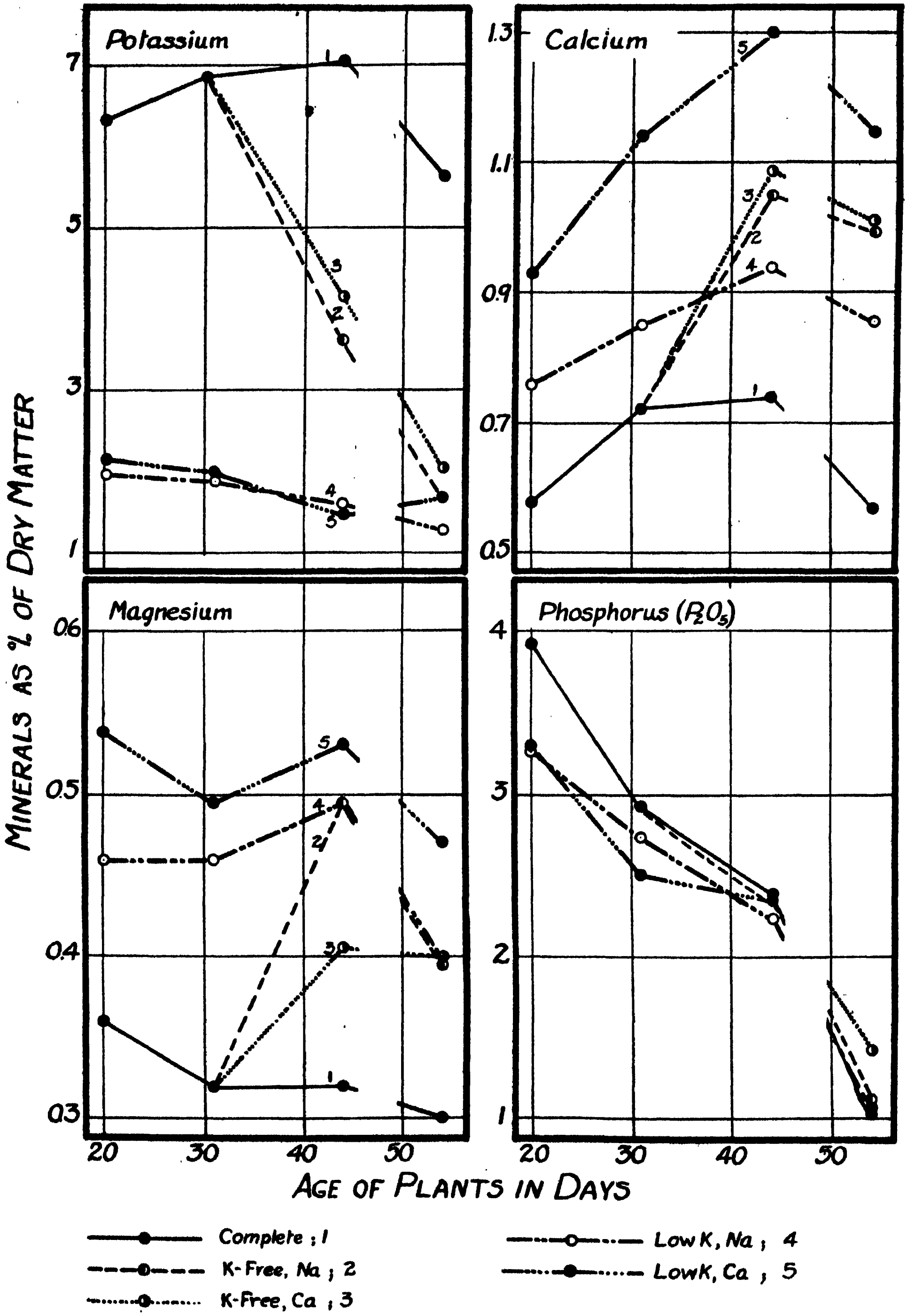

FIG. 4. *The principal minerals expressed as percentages of the dry matter in relation to age of plants and potassium nutrition.*

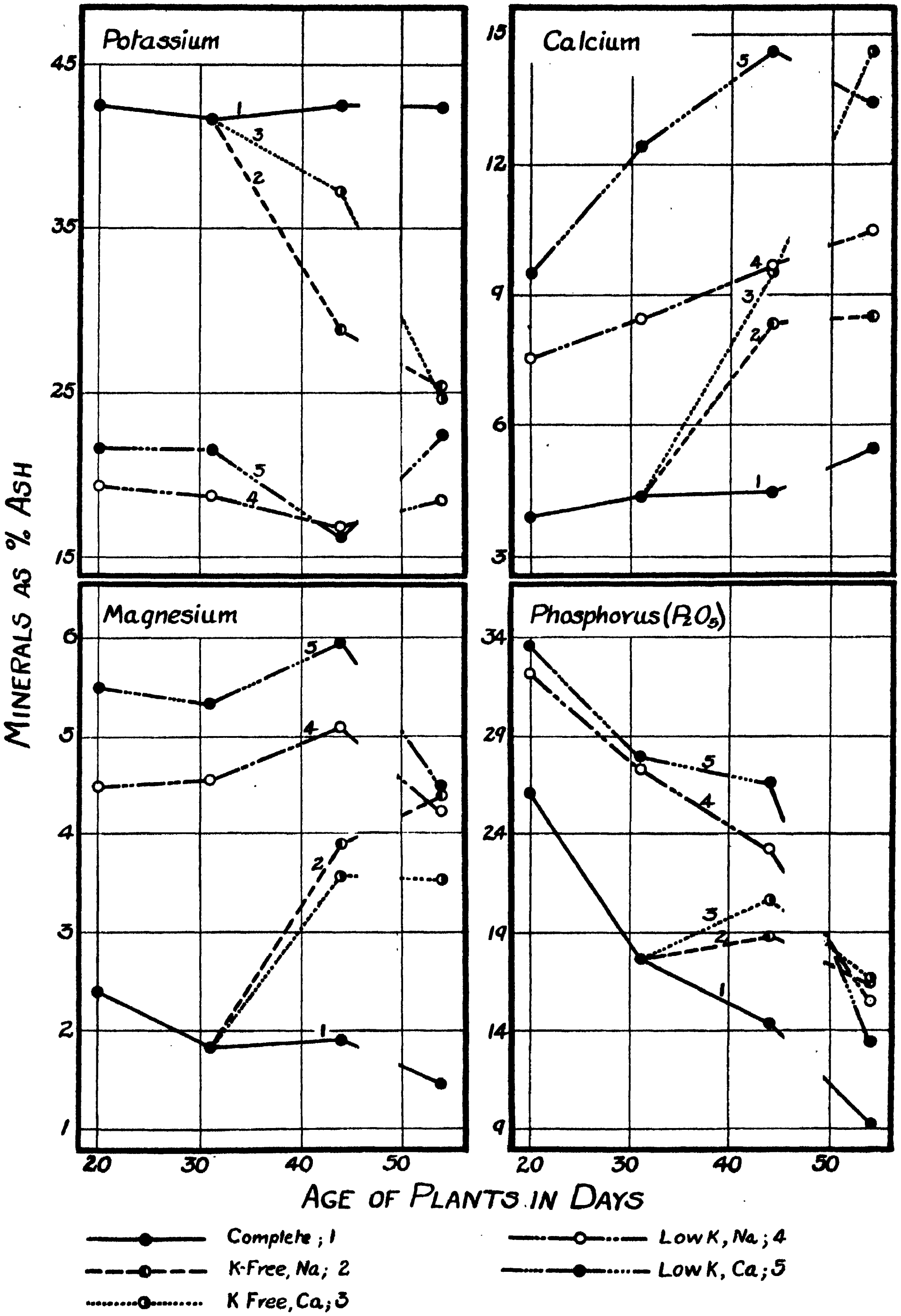

FIG. 5. *The principal minerals expressed as percentages of the ash in relation to age of plants and potassium nutrition.*

The effect of limiting potassium supply was reflected in the calcium and magnesium content of the dry matter and ash. The complete culture plants were decidedly the lowest in their content of both these cations, while the plants grown in the low potassium, sodium solution absorbed considerably less calcium and slightly less magnesium than did those grown in the low potassium, calcium solution. The plants grown in Cultures 2 and 3 showed a rapid rise in the percentage of calcium and magnesium during the first two weeks after the transfer from the complete solution. Sodium seemed to be less effective in replacing potassium in these plants than in those supplied with low potassium solution at all stages.

The absorption of phosphorus was less affected by the modification of potassium supply than was the absorption of other mineral elements. On the basis of percentage dry matter there were but small differences in phosphorus content of the various plants, although those grown in the complete culture were higher during the early stages. On the basis of percentage ash, however, the phosphorus content of the complete culture plants was definitely lower than that of the plants grown in low potassium solutions. When the plants were transferred from the complete to potassium-free solutions, the phosphorus content of the ash increased, and at the time of the final collection it was higher in these plants than in any others. If the phosphorus results are expressed as percentages of the potassium-free ash, the differences among the cultures are greatly reduced, but are still marked.

The restriction of potassium therefore resulted in a definitely increased proportional absorption of calcium and magnesium both in relation to dry matter and total ash, and in an increased absorption of phosphorus in proportion to total ash but not in proportion to dry matter.

Mature plants. The amounts of the various ash constituents present in the mature plants are given in Table IV. The ash values were all higher than in the earlier experiment (9). The difference between the ash uptake of the complete and that of any one other culture was more than the difference in the uptake of potassium, indicating that the general absorption of mineral constituents was impaired by potassium restriction.

In all but the complete culture plants, potassium absorption was largely determined by potassium supply. Although, as already stated, the plants grown in the complete : potassium-free, sodium culture contained the least potassium per 100 gm. of dry matter, this culture absorbed more during the first month of development in the complete solution than did the low potassium cultures throughout their growth.

More calcium per 100 gm. of dry matter was absorbed by the low potassium, calcium culture than by any of the others, but the other restricted potassium cultures were lower than the complete. This was quite a different situation than with the developing plants where the percentage of calcium was lowest for the complete culture. Obviously, these plants absorbed much more calcium after heading began than did the plants in the other cultures. This demonstrates the effect which the supply of one element may have on the absorption of another. Despite the differences in relative absorption, the complete culture plants absorbed more, and the low potassium, calcium plants less, calcium than did the other two cultures.

As in the previous experiment, the differences in the magnesium content were smaller than those for potassium and calcium, but the complete culture appeared to be significantly lower and the complete : potassium-free, sodium culture higher than the others.

The phosphorus results were similar to those for magnesium in that the complete : potassium-free, sodium culture was the highest both in weight per 100 gm. of dry matter and in total absorption. Complete absence of potassium apparently induced an increase uptake of phosphorus in proportion to the total production of dry matter. In the earlier experiment (9) high phosphorus absorption accompanied high calcium uptake, but in the present work, the phosphorus content of the culture in which calcium replaced potassium was no higher than that of the check.

The magnesium and phosphorus results for developing and mature plants differed as did the nitrogen and calcium results. Undoubtedly they were also affected by the absorption of these elements after vegetative growth had practically ceased or heading had started.

Although there are minor differences between these and previous results, the general conclusion that restricted potassium supply decreases total absorption is substantiated. The conclusion that the composition of the culture solution does not wholly determine the composition of the plants is also borne out. The selective absorption noted in the previous experiment was again manifested, for although the ratio of potassium to calcium in the low potassium solutions was as low as 1:50, the ratio of absorbed potassium and calcium was approximately 1:1.

The general development of the plants in the present experiment was not as good as in the previous year. The late start and consequent subjection to hot summer conditions during the early stages of development probably caused the lower dry weight per plant, but is believed to have had no marked influence on the differential behavior of the cultures.

It should be noted that the restriction of potassium supply in this experiment not only affected chemical composition but definitely retarded growth (Table V). The substitution of sodium for potassium was less deleterious than the substitution of calcium, whether the plants were grown in the low potassium solutions at all times or transferred to potassium-free solutions after having an unrestricted supply for one month. Sodium, however, did not compensate for the low potassium either in maintaining growth or in absorption. These results are in good agreement with other work on absorption and the effect of the replacement of one ion with another (4).

Yield and Grade

The results for yield per tank, grade and weight of grain are presented in Table V. The sample (Culture 4) with the highest grade also had the highest weight per bushel and weight per thousand kernels. The grain from this culture in the previous experiment had very small kernels, with high grade and weight per bushel. The results afford no explanation as to why the grain from this low potassium, sodium culture should have differed so markedly in the two years.

TABLE V

YIELD, GRADE AND WEIGHT OF MATURE MATERIAL

Culture No.	Description	Yield, gm. per tank			Grade of grain	Weight per bushel, lb.	Weight per 1000 kernels, gm.
		Straw	Grain	Total			
1	Complete	539	152	691	1 Northern	61.5	27.24
2	Complete : K-free, Na	467	204	671	1 Northern	61.5	29.61
4	Low K, Na	308	173	481	1 Hard	63.0	33.58
5	Low K, Ca	170	83	253	1 Northern	61.5	25.53

The yield results are particularly striking as they show that the total dry matter produced by the complete culture was the highest, but that the weight of grain from this culture was less than from the two in which restricted potassium was replaced by sodium. This lower proportion of grain to straw in complete culture plants has been noted before (8, 9), as has the fact that it is accompanied by a shrivelling of the kernels. The uptake of nitrogen after heading was considerably greater in this than in the other cultures, and the high nitrogen absorption, taking place after the dry weight has reached a maximum, apparently results in a shortage of soluble carbohydrates for translocation to the developing grain (8). This results in a high nitrogen content of the kernels, but a reduced yield of grain. This effect has been obtained in three distinct experiments, and seems to be fully established.

The low yield from the low potassium, calcium culture indicates that substitution of calcium for potassium was more deleterious than a similar substitution of sodium. The fact that the complete:potassium-free, calcium culture failed to develop normally supports this conclusion. The solutions in which calcium was substituted for potassium contained no univalent cation, and it is possible that the beneficial effect of sodium as compared with calcium may be an effect of maintaining a physiologically balanced solution.

Composition of Grain

The results of the analyses of the grain are presented in Table VI.

Nitrogen as a percentage of dry matter of the grain was closely related to the amount of this element absorbed per 100 gm. of dry matter. This fairly constant proportional translocation of nitrogen to the kernels has been discussed before (9), and the present results support the conclusion that regardless of the amount of total nitrogen absorbed, about 75% of it will be found

TABLE VI

Composition of grain as percentage of dry matter

Culture No.	Description	N	Ash	K	Ca	Mg	P_2O_5
1	Complete	3.34	2.75	0.74	0.10	0.21	1.29
2	Complete : K-free, Na	2.83	2.40	0.54	0.08	0.20	1.19
4	Low K, Na	2.71	2.32	0.52	0.08	0.21	1 06
5	Low K, Ca	3.24	2.41	0.64	0.11	0.21	1.38

in mature kernels, and the percentage nitrogen content of the kernels will be determined by the relation between yield and nitrogen absorption. Thus the nitrogen content of the grain from the low potassium, calcium culture was almost as high as that of the grain from the complete culture, although the total absorption and yield were much lower.

The ash content of the grain also showed a rough agreement with the absorption by the whole plant, but this relation was less marked than with nitrogen. There was a selective translocation which operated to keep the mineral composition of the grain more nearly uniform than the composition of the whole plant. The potassium content of the kernels was roughly parallel to the nitrogen content, but the calcium and magnesium results showed little variation. The phosphorus content was also roughly parallel to the nitrogen content, a fact in agreement with previous work (9).

Quality of Grain

In the previous experiments, quality was estimated by studying the distribution of nitrogen in the grain, and by examining the physical properties of the gluten. In the present experiment these methods were supplemented by a baking test carried out on finely ground, whole-wheat meal.

The results for nitrogen distribution are presented in Table VII. The outstanding feature of these results is that the grain from the low potassium, calcium culture contained markedly less gluten nitrogen in proportion to total nitrogen than did the grain from the other cultures. The value 1.06% of the dry matter as non-gluten nitrogen is higher than has been previously obtained by the writers with widely varying material, and demonstrates the failure of the lower nitrogen compounds to condense to form gluten.

TABLE VII

Distribution of nitrogen in grain

Culture No.	Description	Total nitrogen, % D.M.	Nitrogen in gluten		Nitrogen washed from meal	
			% T.N.	% D.M.	% T.N.	% D.M.
1	Complete	3.34	79.0	2.64	21.0	0.70
2	Complete : K-free, Na	2.83	73.6	2.08	26.4	0.75
4	Low K, Na	2.71	78.3	2.12	21.7	0.59
5	Low K, Ca	3.24	67.4	2.18	32.6	1.06

The physical characteristics of the gluten are recorded in Table VIII. The highest yield of wet gluten was obtained from the grain grown in the complete solution. Although the results are not very definite, the lower dry matter content of this gluten suggests that it is of better quality than the others. More extensive studies have shown that better quality gluten usually retains more water than does coarser, more open gluten. The gluten from the grain grown in the low potassium, calcium culture had the poorest quality of the four studied.

TABLE VIII

PHYSICAL CHARACTERISTICS OF GLUTEN

Culture No.	Description	Weight of wet gluten, gm. per 10 gm.	Dry matter in gluten, %	Nature of wet gluten			
				Elasticity	Extensibility	Spring	Color
1	Complete	6.75	33.5	Very good	Good	Very good	Very good
2	Complete : K-free, Na	5.80	35.0	Fair	Fair	Fair	Fair
4	Low K, Na	5.90	34.3	Good	Good	Good	Poor
5	Low K, Ca	6.14	34.4	Fair	Poor	Fair	Fair

The wheat-meal baking test was carried out by baking a mixture of 75% whole wheat meal with 25% of a weak pastry flour, using 50-gm. samples and the malt-phosphate-bromate formula described by Aitken and Geddes (1). The details of this method will be published later when more extensive tests have been carried out. Preliminary work has shown that this procedure gives results in good agreement with those obtained when flour milled from the same sample is baked. The method has been used with several series of named varieties of hard wheat, and has consistently placed the varieties in their accepted order for quality. Calculation of the deviation between duplicates has shown that a difference of 14 cc. between the volumes of two loaves is significant.

The baking results obtained from the present series show that the grain from the complete culture plants yielded a loaf at least 25 cc. larger than that baked from the grain produced by any of the other cultures. There were no significant differences among the other results, but there seems to be no doubt that restricting potassium supply decreases the strength of the wheat as measured by the volume of the bread.

TABLE IX

BAKING TEST

Culture No.	Description	Loaf volume, cc.	External form	Crumb texture
1	Complete	309	Good	Good
2	Complete : K-free, Na	276	Flat	Fair
4	Low K, Na	282	Flat	Fair
5	Low K, Ca	284	Very good	Good

The weakness in external form and texture of the samples from the cultures in which sodium was used was expected from the results of the study of the gluten, but the form and texture of the bread baked from the other restricted potassium sample were better than anticipated.

The quality studies in general show that the grain from plants grown in the complete solution was superior to the other samples, but do not permit definite differentiation among the latter. Had the growth of the plants produced in the low potassium, calcium culture not been impaired to such an extent that the nitrogen content of the grain was higher than that of the other limited potassium cultures, there is reason to believe that the quality would have been lower than in the cultures where sodium was used to replace potassium. This was the conclusion reached as a result of the first year's study.

Discussion

The results of this experiment are in agreement with the previous year's work. Limiting potassium supply retarded growth, reduced the uptake of inorganic elements and greatly altered the chemical composition of the whole plant. It also had a harmful effect on quality, although the composition of the grain was not altered as much as the composition of the rest of the plant. Neither sodium nor calcium was effective in replacing potassium, but sodium was the better in this respect.

In interpreting results of an analytical study such as this, care must be taken to consider both percentages and total weights. The importance of this point has been discussed by Bartholomew, Watts and Janssen (2), who point out some of the erroneous conclusions reached as a result of the study of percentage data only. Hibbard and Grigsby (5) considered only percentage data in interpreting their results on nitrate accumulation in pea plants grown in potassium-deficient solutions. These plants contained more nitrate than did similar plants grown in a complete solution, although the percentage of total nitrogen was approximately the same in both cases. Knowing that there was an abundant supply of carbohydrates in both deficient and normal plants, the authors concluded that the higher nitrate content was due to a greater nitrate absorption. If the total weight of nitrogen absorbed is calculated, however, it is found that in every case the complete culture plants absorbed more nitrogen than the deficient plants. Since all the nitrogen was supplied as nitrate, the higher nitrate concentration could not be due to a greater absorption, and was in all probability caused by a failure of the nitrate-reducing mechanism of the deficient plants.

The effect of potassium nutrition on the course of nitrogen metabolism has been studied by many workers, but the results are not in good agreement. It has been variously reported that the percentage nitrogen content of potassium-deficient plants is lower (4), higher (7) and the same (5) as that of complete culture plants. This percentage of nitrogen is obviously determined by the relation between total growth and total nitrogen absorption. In the present experiment, Culture 5 was so retarded in growth, by restricting the potassium supply, that the percentage of total nitrogen was almost as high as that of the plants grown in the complete culture, although the total absorption was less than one-third. This effect was more accentuated in Culture 3, which was discarded because of the failure to produce seed, and the percentage of total nitrogen was actually higher than that of the check, although the total absorption was lower than that of any of the other cultures. In Cultures 2 and 4, where the limitation of potassium did not retard growth as much, the percentage of nitrogen was considerably lower than that of the checks. Thus the disagreement among the results of other workers may possibly be explained by the relative effects of potassium deficiency on growth and nitrogen absorption.

Just as the effect of nutrition on the metabolism of the plant cannot be determined by the study of percentage data alone, neither can the effect of nutrition on the quality of the grain be studied by analyzing only the grain. This point was discussed in the earlier paper, and the results of the present experiment are in agreement. The effects of nutritional disorders are often manifested to a much greater extent in the composition of the straw than of the grain, and if reliable conclusions are to be drawn from this type of study the composition of the grain in relation to the rest of the plant must be considered.

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STUDIES ON THE NATURE OF DISEASE RESISTANCE IN CEREALS

III. THE ORGANIC NITROGEN CONTENT OF MATURE AND IMMATURE TISSUES OF THE WHEAT PLANT IN RELATION TO STEM-RUST RESISTANCE[1]

By T. Johnson[2] and O. Johnson[3]

Abstract

Analyses were made to determine the organic nitrogen content of immature and mature tissues of six wheat varieties at a stage of growth about a week prior to the emergence of the flag leaf. The immature tissues comprised the leaves folded within the uppermost sheaths, while the mature tissues were represented by the fully grown leaves of the upper half of the plants. In all the varieties the nitrogen content of the mature tissues was greater than that of the immature ones. The greater susceptibility of the younger tissues can not, therefore, be attributed to a higher organic nitrogen content.

Introduction

The young, rapidly developing tissues protected by the sheaths of the growing wheat plant have been shown by Newton and Brown (3) to be more susceptible to stem rust (*Puccinia graminis tritici* Erikss. and Henn.) than exposed, fully grown plant parts. Analyses for sugar content (2) showed that the young tissues (leaves and immature stem parts still enclosed by the sheaths) were considerably richer in sugars than the older plant parts, that is, than the fully grown, but still photosynthetically active, leaves and sheaths.

It has been recognized for some time that nitrogenous fertilization is capable of increasing rust development on cereal plants. Recently, Gassner and Franke (1) have attributed this phenomenon to an actual increase in the nitrogen content of the plants. In moderately resistant cereal plants they found that an increase in the nitrogen content led to an increased susceptibility to rust. Accordingly they maintain that an excess supply of nitrogen increases the nitrogen content, and consequently the protein content, of the host plants and thereby makes available to the rust organism a greater amount of the protein elements requisite for its development.

The present authors have analyzed mature and immature tissues of the wheat plant to ascertain whether or not the immature tissues, which are more susceptible to stem rust, are richer in organic nitrogen than the more resistant, mature tissues. If such were shown to be the case there would be reason to suppose that the higher nitrogen content was a factor contributing to the greater susceptibility of the immature tissues.

Six wheat varieties were selected for analysis. On the basis of rust reaction these varieties may be divided into three groups. (i) Hope, R.L. No. 716,

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[1] *Manuscript received October 26, 1935.*
Contribution No. 439 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

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and Pentad possess the so-called "mature-plant resistance". In the seedling stage they are moderately resistant or even susceptible to many physiologic forms but develop a high degree of resistance as they approach maturity. (ii) Iumillo in all stages of growth shows high resistance to stem rust. (iii) Marquis and Little Club may be classed as susceptible, the latter variety being susceptible to the great majority of the known physiologic forms of wheat stem rust. In all these varieties, with the apparent exception of Iumillo, the rapidly growing tissues protected by the sheaths are susceptible to physiologic forms to which their more mature plant parts are resistant. In Iumillo even the younger tissues appear to be highly resistant.

Methods

The six wheat varieties were grown in the field in rod rows. Material was collected for analysis at least one week prior to the emergence of the flag leaf —at a stage when most of the culms, including tillers, bore four or five leaves. All collections were made between 1:00 and 2:00 p.m. The young tissues comprised the leaves folded within the uppermost sheaths of the plants while the mature tissues were represented by leaves fully grown at the time of collection. These plant parts correspond to those previously analyzed for sugar content (2). The method of collecting the two types of tissue was the same as that employed in the earlier work, the leaves being stripped off and dropped into a covered glass jar lined with moist blotting paper, while the young tissues were separated out rapidly and dropped into a similarly prepared glass receptacle. Two 15-gm. samples of young and of mature tissue of each wheat variety were then dried rapidly in an "air-blast" oven at 85° C., after which the dried material was used for analysis. An analysis of the duplicate samples of both types of tissue was carried out according to the Kjeldahl method for organic and ammonia nitrogen as given in the A.O.A.C. p. 21, 1930. The results are given in Table I.

Experimental Results

Table I shows that in all the varieties tested the nitrogen content of the older tissues is greater than that of the younger. The greater susceptibility of the latter tissues to rust can not therefore be attributed to a higher organic nitrogen content. It follows that the above-mentioned conclusions of Gassner and Franke are not applicable in the case of the tissues under discussion unless it is assumed that the organic nitrogen of the younger tissues is present in a form more utilizable to the fungus than that of the older plant parts. There is, of course, a possibility that the greater nitrogen content of the older tissues fails to induce susceptibility to rust through interference by some unknown factor such as the development, in the more mature tissues, of a substance inhibitory to the growth of the rust organism. It does not seem probable, at any rate, that the resistance to rust developed by the more mature tissues of the wheat plant can be explained on any purely nutritional basis.

TABLE I

PERCENTAGE OF ORGANIC NITROGEN IN YOUNG AND OLDER TISSUES OF WHEAT VARIETIES

Wheat variety	N as % dry weight		N as % green weight	
	Immature tissues	Mature tissues	Immature tissues	Mature tissues
Hope	2.60	3.85	0.403	0.770
	2.58	3.85	0.400	0.770
R. L. No. 716	2.82	3.64	0.400	0.870
	2.84	3.58	0.409	0.824
Pentad	2.44	3.01	0.349	0.542
	2.35	2.96	0.336	0.553
Iumillo	2.64	3.73	0.375	0.627
	2.56	3.55	0.369	0.649
Marquis	2.70	3.60	0.400	0.742
	2.68	3.82	0.402	0.796
Little Club	2.60	4.03	0.369	0.649
	2.46	3.71	0.335	0.671

Acknowledgment

The authors are indebted to the Department of Chemistry of the University of Manitoba for the use of its Kjeldahl apparatus.

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MICROBIOLOGICAL STUDIES OF APPALACHIAN PODSOL SOILS

II. SEASONAL CHANGES IN MICROBIAL ACTIVITY[1]

By P. H. H. Gray[2] and H. J. Atkinson[3]

Abstract

Seasonal fluctuations in numbers of micro-organisms in soil, estimated by the plate method for bacteria and actinomyces and by the evolution of carbon dioxide, have been studied with samples of cultivated podsol soils, drawn from experimental fields under various treatments at three farms in the Eastern Townships region of Quebec, at intervals of six weeks during the cropping season in two years. The nature of the fluctuations in numbers of micro-organisms was similar in the three soils, which were situated many miles apart. In 1931 numbers were lower in summer than in spring and autumn; in 1932 numbers fell towards the end of the season. The evolution of carbon dioxide fluctuated to a comparatively less extent than the bacterial numbers, but there was general agreement in the direction of the changes in the two years. Biological activities in any one soil were at significantly different levels in the two years. There was an agreement between numbers of micro-organisms and the amount of carbon dioxide, in that changes in both of these factors were in the same direction in the second year of sampling; there was also evidence of agreement between intra-annual changes in numbers and carbon dioxide, especially in the alkali-treated plots. Fluctuations in numbers and carbon dioxide in samples from plots in different parts of the same field or farm were in conformity, and were superimposed on the effects of chemical treatments that caused significant increases in microbial activity.

Introduction

The fluctuations of bacterial numbers in field soil, at short intervals of time, have been found to be of sufficient amplitude to make isolated determinations of numbers valueless except for comparisons of such treatments as can be shown to disturb the normal equilibrium of the soil population (19). It may, nevertheless, be true that the difference in general level of numbers between two seasons of the year may be sufficiently great to be accepted as significant and as being due to factors inherent in a changing microflora. The acceptance of these changes as due to causes other than those of random sampling can be justified only after a study of a sufficiently large number of samples.

The changes of bacterial numbers found by Cutler, Crump and Sandon to occur daily in field soil supply the most reliable evidence of intra-annual and inter-annual changes, to which, however, no adequate causes could be ascribed (2). It is clear, from their graphs of fortnightly averages, that the bacterial numbers at midsummer, 1921, were about double those found at midsummer, 1920. Similar inter-annual differences in bacterial numbers in field soils were found by Crump in samples taken at weekly intervals during 1916 and

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Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que., Canada. Macdonald College Journal Series No. 68. This paper is the second in a series dealing with part of the work of the Soil Fertility Committee of Macdonald College, the names of the members of which were given in the previous paper (Macdonald College Journal Series No. 63, in the Canadian Journal of Research).

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1917 (1); in this case, however, it was suggested that soil moisture could be accepted as one of the causes contributing to the intra-annual (weekly) changes (6). The work of Erdman, in Iowa, also showed that numbers of bacteria may be at considerably different levels in the same season of two years (4).

Newton found that bacterial numbers in Alberta soils fluctuated extensively, and confirmed the general view that numbers are lower in summer than in spring and autumn; the results also suggest that the average numbers in two years were distinctly different (10). The results of Gräf, though not based on an extensive series of counts, are in conformity, in that numbers fell during the summer, and are of added interest in that the numbers near to and away from plant roots, though at considerably different levels, were affected similarly by the season (5). This statement applies to the normally mixed population of colonies appearing on a moderately selective medium. On the other hand, she reported that the numbers of a specific group, *Radiobacter*, increased rapidly during July and August.

The seasonal fluctuations of numbers and activity in specific groups of micro-organisms have been demonstrated conclusively by the use of partially selective mediums; less selective mediums tend to mask the fluctuations that may exist in smaller groups (18). In this connection the results of Vandecaveye and Anderson are of much interest; they found that *Azotobacter* was present in soil in varying numbers in spring, vanished in June, July and August, and returned to show further seasonal fluctuations until November in one year (20). They suggested that although this organism can be shown to fluctuate extensively in regard to numbers, the numbers counted may not be contributing anything to the soil's store of fixed nitrogen, at least not until their numbers reach a much higher level than the maximum reported to have been reached that year. This is a point of importance in any discussion on the probable significance of changes in numbers in specific groups of micro-organisms.

The numbers of bacteria in a soil are to some extent dependent upon the presence of higher plants, and the distance from the latter at which a sample is taken, as was shown by Gräf in the work referred to above. The work of others has been reviewed by Starkey, who gives further evidence of the effects of living plant roots on the numbers of micro-organisms and on the evolution of carbon dioxide during one growing season in which fluctuations occurred in both fallow and cropped soil. The best evidence of seasonal fluctuations in bacteria and other micro-organisms rests upon results of sampling fallow soil (2, 19), or soil now or recently under grass (3, 16). In a previous paper the present authors have shown that when the method of sampling adopted in this work was used, the numbers of bacteria in cropped plots receiving no treatment were not different from those in fallow plots (7). The results submitted below would seem to suggest that seasonal fluctuations may be superimposed on changes due to treatments which are even more efficient in their effects upon the microbial equilibrium than is the growth of plants.

The same considerations apply to fluctuations in the evolution of carbon dioxide by soil micro-organisms, determined by methods that take into account or eliminate the possible influence of growing plants. A large proportion of the carbon dioxide in soil air is due to micro-organisms decomposing the organic residues, for comparisons of fallow and cropped soil seem to indicate little or no significant differences (7, 17). Seasonal changes that can most reasonably be ascribed to micro-organisms have been reported to occur intra-annually by Jodidi and Wells (8), Russell and Appleyard (12, 13), Potter and Snyder (11), and by Smith, Brown and Millar (15). The results of Russell and Appleyard are of interest in the present work, because these workers determined the respiratory intensity of soil in manured and unmanured field plots for two years, and in several instances determined both bacterial numbers and amount of carbon dioxide on the same day, though only in the latter half of the two summers, *viz.*, July 11 to Sept. 22, 1913; July 7 to Sept. 21, 1914. The amounts of carbon dioxide decreased considerably between the dates mentioned, the results for the two years being parallel, and there was slightly more activity in the second year. The results of Potter and Snyder show the need for continuous rather than intermittent sampling; their results from thirteen plots, when averaged, show clearly the differences that might be obtained by taking samples at intervals shorter than six weeks.

The present work was planned to determine the extent of microbial activity in soils more widely separated than it has been usual to select, to ascertain if such intra-annual and inter-annual fluctuations would prove to be local or to occur in a larger area under similar climatic conditions.

Experimental

The soils selected were three that fall into the group of heavily leached (podsol) soils of the Appalachian uplands of Quebec, certain characteristics of which have been described (9, 14); further details relative to the actual soils used are submitted below. The figures in Table I represent the average

TABLE I

SOME CHARACTERISTICS OF THE SOILS UNDER INVESTIGATION

(Figures, except those for lime requirement and pH, represent percentages on a basis of moisture-free soil.)

	Brome County	Compton County	Sherbrooke County
Lime requirement (lb. CaO per acre)	4117	5095	6183
pH	5.46	5.41	5.37
Moisture	1.99	2.61	2.77
Loss on ignition	7.81	9.08	9.74
Carbon (organic)	3.37	4.09	4.44
Total nitrogen	.247	.284	.282
Phosphorus (P_2O_5)	.294	.162	.141
Silica (SiO_2)	72.17	72.36	73.43
Calcium (CaO)	.97	.54	.62
Magnesium (MgO)	.77	.57	.68

values of composite samples of 60 cores each. These were taken to a depth of 6 inches, from the normally ploughed half of the experimental acre at each farm, and sampled as described previously, before treatments had been applied in 1931. The situation of the three farms was shown in the map in the former paper (7).

The effects of certain physical and chemical treatments on biological activity in these selected soils have been described in the preceding paper in this series. The results from those experiments are used in more detail in this paper for a consideration of seasonal changes in microbial activity in samples taken at intervals of six weeks from any one place during the cropping seasons of two years, and from one of the places for three years. The numbers of bacteria and actinomyces were estimated by means of Thornton's mannitol-asparagine medium, and the biological activity in samples was determined by the evolution of carbon dioxide. The average values from the plots started in 1931, at the four sampling periods of the two years, are shown in Tables II and III, and in Figs. 1–4. The values relating to the plots receiving limestone

TABLE II*

NUMBERS OF BACTERIA AND ACTINOMYCES
(MILLIONS PER GM.)
(Averages of 6 plots of 1931)

		1931			*1932*
		Brome County			
April	28	15.313	April	26	11.724
June	9	**9.983**	June	7	12.201
July	21	11.356	July	18	15.182
Sept.	1	**18.061**	Aug.	31	11.443
Mean of 48 samples		13.681			12 637
		Compton County			
May	13	17.199	May	10	7.047
June	23	**10.307**	June	21	9.158
Aug.	4	12.083	Aug.	2	**16.855**
Sept.	15	12.878	Sept.	13	**7.458**
Mean of 48 samples		13.116			**10.130**
		Sherbrooke County			
May	28	11.460	May	24	22.786
July	6	11.883	July	5	21.705
Aug.	18	12.457	Aug.	16	22.740
Sept.	29	**19.960**	Sept.	27	**15.259**
Mean of 48 samples		13.940			**20.643**

Differences required for significance between two values:

Intra-annual 4.406 millions per gm.
Inter-annual 1.981 millions per gm.

TABLE III

EVOLUTION OF CARBON DIOXIDE
(MG. PER 100 GM. PER 100 HR.)
(Averages of 5 plots of 1931)

		1931			*1932*
		Brome County			
April	28	25.68	April	26	17.01
June	9	28.84	June	7	18.91
July	21	31.50	July	18	21.95
Sept.	1	28.62	Aug.	31	17.73
Mean of 40 samples		28.57			**18.90**
		Compton County			
May	13	26.77	May	10	25.04
June	23	32.82	June	21	22.95
Aug.	4	38.31	Aug.	2	21.67
Sept.	15	37.44	Sept.	13	15.01
Mean of 40 samples		33.84			**21.17**
		Sherbrooke County			
May	28	28.57	May	24	60.83
July	6	22.60	July	5	**35.09**
Aug.	18	30.69	Aug.	16	42.68
Sept.	29	29.41	Sept.	27	**32.63**
Mean of 40 samples		27.82			**42.81**

Differences required for significance between two values:

Intra-annual 8.475 mg. carbon dioxide
Inter-annual 4.237 mg. carbon dioxide

**Significantly different values between sampling periods within a year or between the averages for each year, are indicated by bold-face type.*

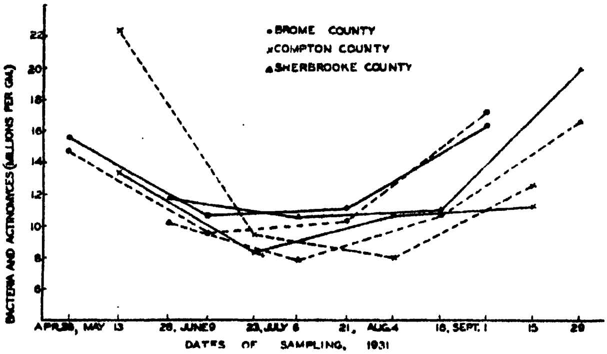

FIG. 1. *Seasonal changes in numbers of micro-organisms in 1931. Normal ploughing, solid line; deep ploughing, broken line.*

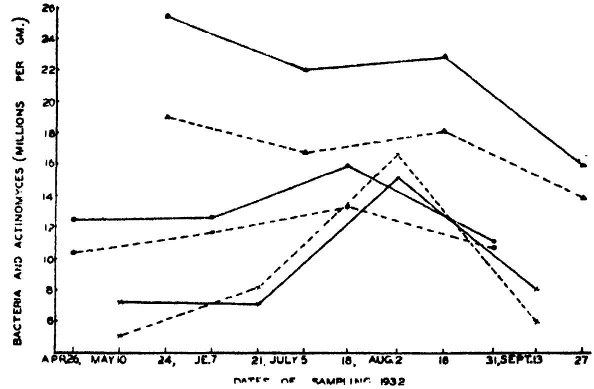

FIG. 2. *Seasonal changes in numbers of micro-organisms in 1932. See Fig. 1 for notations.*

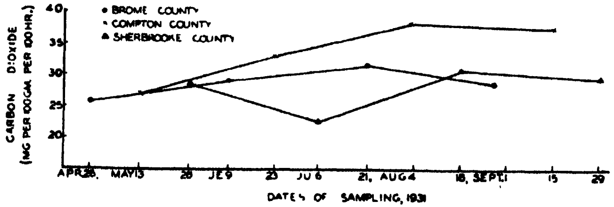

FIG. 3. *Seasonal changes in evolution of carbon dioxide in 1931.*

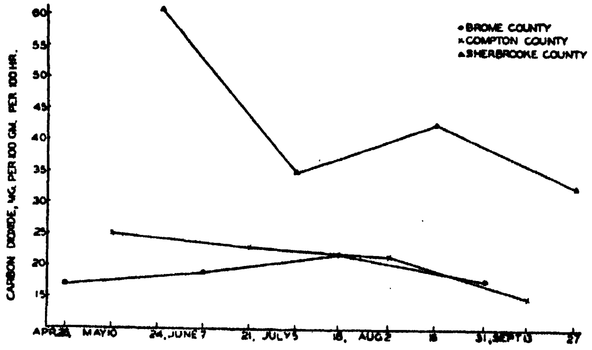

FIG. 4. *Seasonal changes in evolution of carbon dioxide in 1932.*

have not been included in the tables for carbon dioxide, for the reason stated in the previous paper. The significance of a change between one date of sampling and the next, at any one place, has been based on the standard deviations obtained from the analyses of variance in the two biological factors, as previously determined (7).

The results obtained from the small round plots started in 1932 at the same three places, and continued at the Compton County farm in 1933, are shown in Tables IV and V, and expressed graphically in Figs. 5–8; values from the untreated plots have been compared with the average values of the alkali-treated plots. In view of the small size of the plots and the limited scope of these experiments, no attempt has been made to determine the significance of a change between any one date of sampling and the next.

A first consideration in regard to such biological determinations has been to ascertain the reliability of the values obtained. In regard to the numbers of bacteria (including actinomyces) the reliability of the mean value of colonies in from two to five plates was ascertained by use of χ^2; by this analysis it was found that most of the values obtained (about 80% of 455 available plat-

TABLE IV

INTRA-ANNUAL FLUCTUATIONS IN MICROBIAL ACTIVITY
(Experiments of 1932)

Date of sampling	Bacteria and actinomyces, (millions per gm.)		Carbon dioxide, (mg. per 100 gm. per 100 hr.)	
	Control plot*	Treated plots**	Control plot*	Treated plots**
	Brome County			
April 26	6.02*		51.0*	
June 7	16.50	45.01	44.5	63.8
July 18	41.77	53.85	60.9	74.0
Aug. 31	12.72	25.49	41.5	46.1
	Sherbrooke County			
May 24	19.29		34.0	
July 5	13.78	27.02	31.3	46.1
Aug. 16	21.17	35.85	40.7	52.1
Sept. 27	22.80	33.73	31.7	37.4

**Mean of six plots at first sampling.*

***Mean of eleven plots having different treatments of CaO, NaOH, Na_2CO_3.*

TABLE V

SEASONAL FLUCTUATIONS IN MICROBIAL ACTIVITY
(Compton County—Experiment of 1932)

Date of sampling	Bacteria and actinomyces, (millions per gm.)		Carbon dioxide, (mg. per 100 gm. per 100 hr.)	
	Control plot*	Treated plots**	Control plot*	Treated plots**
	1932			
May 10	4.56*		27.6*	
June 21	8.12	43.05	18.9	39.1
Aug. 2	10.92	35.72	34.8	34.5
Sept. 13	5.71	21.56	19.7	30.0
Average	8.25	33.40	24.5	41.2
	1933			
May 18†	10.35	22.17	18.9	35.0
June 13	32.00	39.67	32.9	31.2
July 25	10.35	30.06	36.8	45.8
Aug. 31	16.38	29.18	29.7	37.7
Average	17.27	30.27	29.6	37.4

**Mean values from six plots.*

***Mean values from eleven plots treated with CaO, NaOH, Na_2CO_3.*

†In Figs. 5-8 this date is shown, by mistake, as May 30.

ings) have been based on reliable samples. It may also be stated that in nearly all cases in which the χ^2 value was found to be excessive or subnormal the numbers of organisms calculated from these plates were not greatly different from the numbers calculated for the parallel samples taken at the same time. The analyses for carbon dioxide were based on readings from single composite samples, usually of 500 gm. each, the gas evolved having

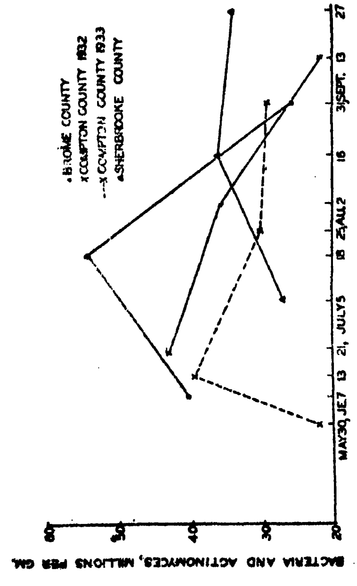

FIG. 5. *Seasonal changes in numbers of micro-organisms in alkali-treated plots of 1932.*

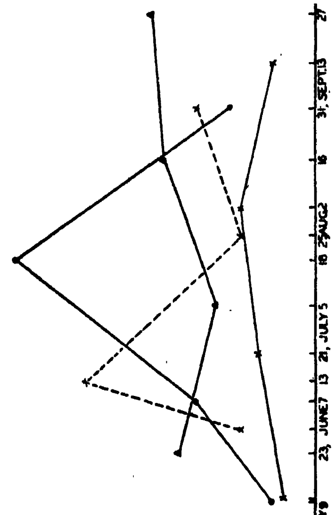

FIG. 6. *Seasonal changes in numbers of micro-organisms in untreated plots of 1932. For notations see Fig. 5.*

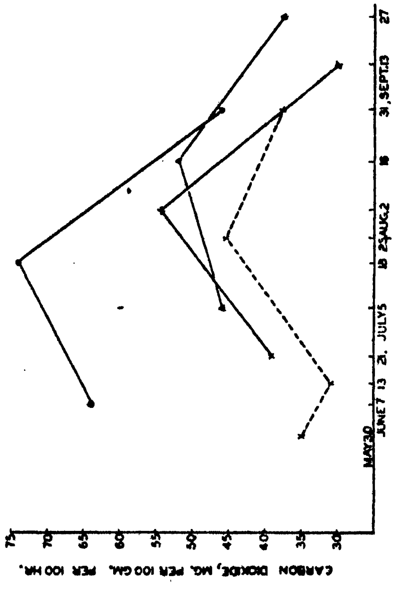

FIG. 7. *Seasonal changes in evolution of carbon dioxide in alkali-treated plots of 1932. For notations see Fig. 5.*

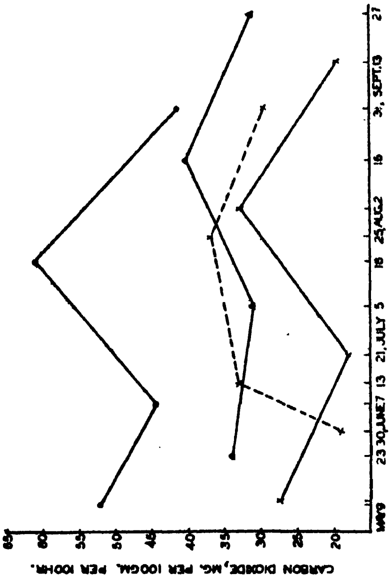

FIG. 8. *Seasonal changes in evolution of carbon dioxide in untreated plots of 1932. For notations see Fig. 5.*

been collected at intervals during an average aeration time of about 310 hr. The interval between collecting the soil sample in the field and starting the aeration into the train of baryta tubes was rarely greater than 24 hr.

Discussion of Results

In the results obtained from the experiments started in 1931 to test the effects of single fertilizers and limestone, the existence of a significant change in numbers or activity between one date and the next has been indicated by bold-face type in Tables II and III. If however, the results be considered without relation to the statistical analyses but only in regard to the general trend of events, they would seem to indicate that changes in these biological factors occurred regularly in the same direction during a season at all of the places. In regard to bacterial numbers, the results for 1931 confirm the general view of other workers, namely, that numbers were lowest during the summer. In 1932, however, numbers tended to rise towards the end of summer, to be followed by reductions of considerable extent. Since deep ploughing was shown to cause significant reductions in numbers and biological activity, it is interesting to note that the fluctuations shown here occurred to about the same extent, on the whole, in both the deeply ploughed and the normally ploughed plots; the results for numbers of micro-organisms from the two treatments (averages of 5 plots, excluding those treated with limestone) are shown graphically in Figs. 1 and 2. In both years the changes in numbers in the two half-acres were in the same directions, though in some cases at considerably different levels.

The activity of the whole micro-population as determined by the evolution of carbon dioxide, from the experiments started in 1931, offers little evidence of significant differences, such changes occurring only twice in 1932, in the Sherbrooke soil. There appears, however, to be some agreement in the curves for the Brome and Compton soils, in both years; activity in these two soils differs in the two years, the curves for 1932 being almost reverse images of those for 1931. In the case of the Sherbrooke soil the changes in the two years are somewhat similar, though at significantly different levels. The changes in carbon dioxide in the Sherbrooke soil, also, in 1932, resemble the changes in bacterial numbers; otherwise the curves for the two factors do not seem to be related.

There appears to be a definite relation between bacterial numbers and carbon dioxide, when the average values from the three places for the two years are considered. As shown in Tables II and III, the higher or lower numbers in 1932 are associated with corresponding higher or lower amounts of carbon dioxide; these differences, with the exception of the lower numbers of organisms in the Brome soil in 1932, were found to be significant.

It may be noted here that the exclusion from Table II of the results from the plots treated with limestone, the effects of which might be thought to have considerably masked any intra-annual changes, would make no difference to the conclusions stated above.

The results from the experiments started in 1932, to test the effects of certain alkali treatments, seem to indicate a fair amount of agreement in the direction of the changes, at two of the places in regard to numbers, and at all three places in regard to general activity (see Tables IV and V, and Figs. 5–8). It is interesting to compare these results with those obtained from the larger plots situated no great distance away. The small plots at the Compton County farm in 1933 yielded results that are to some extent conformable with the results obtained from the same soil in 1932, especially in regard to the evolution of carbon dioxide. Bacterial numbers appear to be in conformity only in the treated plots; it must be noted, however, that after treatments had been applied in 1932, the results from the control plots are based on single samples of four cores, while those from the treated plots represent the average of 11 such composite samples; the latter should be, therefore, the better measure of these determinations.

During the course of these experiments, determinations for soil moisture were also made. There was no evidence that the moisture content of the soils affected microbial activity. In regard to soil temperature, for which, as well as for rainfall, almost continuous daily records from the district are available for the three years, non-conformity of the bacterial numbers between years would seem to indicate that soil temperature was not a determinant external factor.

The results from the small plots of 1932 would suggest that seasonal changes in numbers and activity of micro-organisms may be superimposed upon any increases in activity brought about by chemical treatments, even when these were such as to cause highly significant increases in the soil population in individual plots.

Acknowledgment

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THE INHERITANCE OF DELAYED GERMINATION IN HYBRIDS OF *AVENA FATUA* AND *A. SATIVA*[1]

By L. P. V. Johnson[2]

Abstract

The inheritance of germinability was studied in reciprocal crosses between *Avena fatua*, the seeds of which are non-germinable for several months after harvesting, and *A. sativa*, the seeds of which are fully germinable shortly after harvesting. The following conclusions were drawn from studies of the first three hybrid generations: (1) Germinability is genetically dominant over non-germinability (delayed germination). (2) There is strong evidence that germinability is inherited on the basis of three factors of more or less equal potency, one of which is linked with the factor for grain type. (3) At the time of testing, embryos having completely recessive genotypes were non-germinable; embryos having genotypes with one dominant allelomorph were non-germinable, except in rare instances; embryos having genotypes with two dominant allelomorphs (of the same or different factors) were to a very great extent germinable; embryos having genotypes with three or more dominant allelomorphs were germinable. (4) The germinative potentialities of genotypes vary with the time elapsing between harvesting and testing. (5) There is some evidence that the linked factor has a greater germinative potency than either of the non-linked factors.

Introduction

Studies have been made on the inheritance of the character of delayed germination in reciprocal crosses between the common wild oat, *Avena fatua* L. and a cultivated form, *A. sativa* L., variety Victory. The *A. fatua* parent possesses the property of delayed germination, that is, its seeds are not germinable until several months after harvesting; the seeds of the *A. sativa* parent, on the other hand, are germinable very soon after harvesting. Physiological studies on delayed germination in *A. fatua* have been previously reported (5).

As far as is known, only one previous attempt has been made to study the inheritance of delayed germination, although the phenomenon is by no means of rare occurrence among the species of seed plants. In 1923, Garber and Quisenberry (3) reported data on the inheritance of delayed germination in crosses between *A. sativa* and *A. fatua*, but did not attempt to establish a genetic basis for the character. They concluded from their observations "that in crosses between *A. sativa* and *A. fatua* delayed germination is an inherited recessive character and that it is somewhat loosely linked with the *fatua* type of seed articulation." This statement embodies, essentially, the whole genetic knowledge of delayed germination existing previous to the present work.

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Materials and Methods

Reciprocal crosses were made between *A. fatua* and *A. sativa* var. Victory at the University of Alberta, Edmonton, Alberta, Canada, in 1932. Four hybrid seeds were produced which were designated as follows: Cross 8, in which *A. fatua* was the maternal parent; and Crosses 9, 10, and 11, in which *A. sativa* was the maternal parent.

The *A. fatua* parents were random selections from a plot grown especially for crossing work from a seed stock known to possess a high degree of delayed germination. The seeds from which the plot was grown germinated in consequence of having undergone 18 months of after-ripening. Subsequent germination tests of bulked seed from the crossing plot gave 0.0 and 1.5% germination at four and six months after harvesting.

The *A. sativa* parents were likewise random selections from a plot grown especially for crossing work. Bulked seed from this plot tested two months after harvesting gave 100% germination.

The F_1 plants, together with parental material, were grown in the greenhouse under artificial illumination during the winter of 1932-1933.

The seeds from the F_1 plants were sown in the field in May 1933, about three months after harvesting. Only large, perfectly sound, primary grains were used. A very carefully prepared seed bed together with a period of fine weather provided very favorable conditions for germination. Seeds from the plants of both parents, grown with the F_1, in the greenhouse, were sown as checks. Daily notes were taken on the seedling emergence of all material.

In anticipation of the non-germinability of the *A. fatua* check, a plot was sown from the original *A. fatua* seed stock (30 months old) to provide check material. At the time of harvesting the F_2 plants, a number of plants were selected from this plot to be used as checks for the F_2 germination tests.

The F_2 plants were harvested in August 1933, placed in open envelopes, allowed to dry in the laboratory for about ten days, then boxed and shipped to Pullman, Washington, where the material was stored in a laboratory at room temperature (about 21° C.).

Germination tests of F_2 and F_3 plants were made in a thermostat-controlled, electrically heated germinator held at 20° C. Fifty seeds were tested from each plant, only large, mature, sound, primary grains being selected. Folded paper pads placed on large blotters which fitted the germinator trays were used for all tests. These pads were cut from white paper towels to a measurement of 2 × 3¾ inches, a size which accommodated 50 seeds without crowding and permitted the placing of 40 pads upon each germinator tray. The F_2 tests were continued for 14 days and the F_3 tests for ten days, although germination was usually completed in eight days. The water used for moistening the pads was kept in the germinator in order to avoid modifying the temperature conditions of the seeds at the time of watering.

The F_3 was grown at Pullman, Washington, in 1934. A complete generation was grown directly from the seeds of F_2 plants of Cross 8 which were tested at the beginning of March. Sprouted seeds were removed daily from the germinator pads and transplanted to the field. However, in consequence of their genetic constitution, a large number of seeds failed to germinate. It was necessary that these seeds be forced to germinate in order that complete F_3 lines might be grown. This was accomplished by a method which had been previously devised especially for this purpose. After natural germination had ceased, the non-germinated seeds were removed from the germinators, dried thoroughly, scraped over the embryo with a razor, moistened, and returned to the germinator. After this treatment, practically 100% germination occurred. In all, 7,133 seeds out of a total of 7,150 germinated either naturally or by artificial stimulation and were transplanted to the field.

The fact that F_3 lines arising from these transplantings averaged only slightly more than 40 plants each, and the fact that 40 germination pads was the maximum number accommodated by a single germinator tray, led to the limiting of tests of F_3 lines to the seeds of 40 plants.

In both the F_2 and the F_3, all plants studied for germinability were also classified with respect to seed type in order that linkage relationships might be studied.

In all, individual germination tests were made upon 317 F_2 plants and approximately 3300 F_3 plants comprising 84 families.

Experimental Results and Their Analysis

In studying the inheritance of delayed germination we are dealing, primarily, with generations of embryos rather than mature-plant generations. This fact may lead to some confusion, especially since germinability of the embryo is correlated with a mature-plant character and as the experimental results are largely discussed from the point of view of this correlation. It might be well, therefore, to discuss the matter of generation designations before taking up the discussion of experimental results.

The original hybrid seeds possessed F_1 embryos, seeds of F_1 plants possessed F_2 embryos, seeds of F_2 plants possessed F_3 embryos, etc. Thus, when placed under germinative conditions, the seeds of F_1 plants expressed F_2 segregation for embryonic germinability, the seeds of F_2 plants expressed F_3 segregation, etc. In the genetic analyses, however, the average germinability of the seeds of an F_2 plant, for example, is assumed to be equal to the genetic, germinative potentiality of the F_2 embryo carried by the progenitorial seed of the F_1 plant. Thus, when the term F_2 is used, the question of whether an embryo or a mature plant is meant need not arise, for in the analyses the absolute germinability of the embryo and the average germinability of the seeds of the plant derived from the embryo are taken to mean exactly the same thing. Similarly, in the case of the term F_3. We may look upon the embryo and the plant developed from it as the same individual in different stages of development.

THE FIRST GENERATION

The first generation is represented by the embryos developed directly from the fertilizations brought about by hybridization. Four hybrid seeds (F_1 embryos) were produced. *A. fatua* was the maternal parent in one cross, the hybrid line from which has been called Cross 8; *A. sativa* was the maternal parent in three crosses, the hybrid lines from which have been called Crosses 9, 10, and 11.

In germination tests made three months after harvesting, all four hybrid seeds germinated, the seeds of the *A. sativa* parent gave practically 100% germination, while the seeds of the *A. fatua* parent failed to germinate. These results clearly indicate that delayed germination is genetically recessive to the *A. sativa* germinative capacity.

THE SECOND GENERATION

The genetic analysis of the F_2 has two bases: (i) the direct observation of the germination or non-germination of F_2 embryos in tests of the seeds of F_1 plants, and (ii) the average percentage germination of F_3 embryos carried by the seeds of individual F_2 plants. The data compiled from tests of the seeds of F_1 plants will be considered first.

1. Results from the Seeds of F_1 Plants

Seeds of the four F_1 plants, together with seeds from both parents, were sown in the field approximately three months after maturity. Only large, mature, sound, primary grains were used. Daily observations on the emergence of seedlings were made. The results from these observations are summarized in Table I, in which the criterion of seed germination is considered to be the emergence of the seedling.

TABLE I

EMERGENCE DATA FROM SEEDS OF F_1 AND PARENTAL PLANTS

Cross	No. of seeds sown	No. of seeds germinated	No. of seeds non-germinated	Ratio, germinated: non-germinated
Cross 8	152	145	7	20.7 : 1
Cross 9	91	90	1	90.0 : 1
Cross 10	94	90	4	22.5 : 1
Cross 11	137	136	1	136.0 : 1
Total of all crosses	474	461	13	35.5 : 1
A. fatua parent	50	0	50	—
A. sativa parent	50	50	0	—

The observed ratio between the numbers of germinated and non-germinated seeds varies greatly in the different crosses. This variation is, apparently, not correlated with the reciprocal nature of the crosses. None of the observed ratios show a close correspondence with any standard F_2 ratio. Furthermore, the wide deviations among the different observed ratios rule out attempts to fit these ratios, as they now stand, to a hypothetical F_2 ratio.

2. Results from the Seeds of Individual F_2 Plants

The seeds of individual F_2 plants of Crosses 8, 9, and 10 were tested for germinability. The time elapsing between harvesting and testing was three months for Cross 9, five months for Cross 10, and six months for Cross 8. The results of these tests are combined and summarized in Table II.

TABLE II

FREQUENCY DISTRIBUTION OF PERCENTAGE GERMINATION OF INDIVIDUAL F_2 PLANTS OF CROSSES 8, 9, AND 10

Cross	Grain type	Percentage germination of seeds from individual F_2 plants																					No. of plants not tested	Total no. of plants
		0	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	71–75	76–80	81–85	86–90	91–95	96–100		
	w																		1	4	4	30		39
8	I				1	1	1	1		2	1	1	1	5	2	3	1	2	4	5	3	36	2	72
	W				1	1	1			1				1	1		2	7	2	2	8	7		34
	w																		1	1	7	14		23
9	I							1					1	1	3	2	1	4	5	8	9	6	2	43
	W			1		2			1			1						5	1	4	5	2		22
	w											1		1						2	3	15	1	23
10	I							2		2	2	1		2		2	1		4	7	5	17	1	46
	W											2				2		2	4	4	1	6		21
Total				1	2	4	2	4	1	5	3	6	2	10	6	9	5	20	22	37	45	133	6	323
A. sativa (w) parent																						14		14
A. fatua (W) *parent*		13	1																					14

The F_2 plants of these crosses were also classified with respect to type of grain. It has been shown by Aamodt, Johnson and Manson (1) that in crosses between *A. fatua* and *A. sativa* segregation occurs for three grain types, namely, *sativa*, intermediate, and *fatua* types, in the monohybrid ratio of 1 : 2 : 1. As in the previous work, *sativa* types are designated by w, intermediate types by I, and *fatua* types by W. (See Fig. 1). In Table II, the data from germination tests of each cross have been divided on the basis of segregation for these grain types.

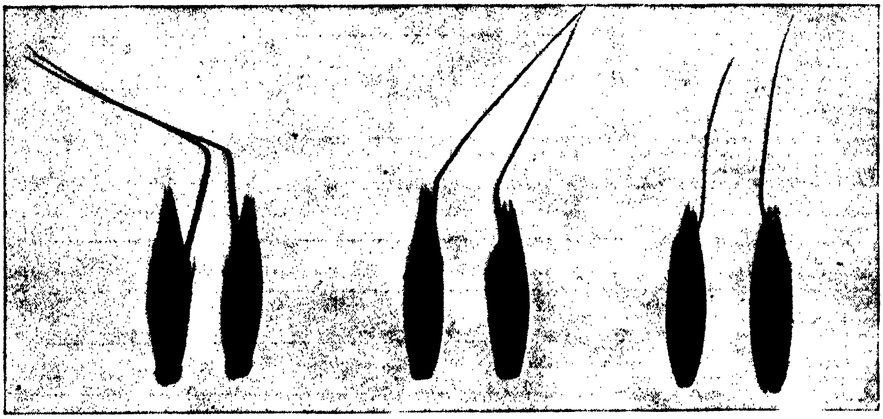

FIG. 1. *Segregation for grain type. Left to right in pairs: fatua (W), intermediate (I), and sativa (w) types.*

Though tested at different periods the germination percentages of the F_2 plants of the different crosses are distributed in essentially the same manner and with similar relative frequencies in the various classes. It seems reasonable to assume, therefore, that at the time of testing, the germinative capabilities of the various genotypes were essentially the same in all crosses. There is no reason to suspect that there was any appreciable germination of seeds possessing the genetic constitution of the non-germinable parent, especially as only one germination occurred among the 700 seeds comprising the 14 *A. fatua* checks. This germination occurred in one of the checks of the Cross 8 tests which was tested six months after harvesting. In view of the above facts, the data from all three crosses are considered to be directly comparable, despite their compilation at different times.

The non-occurrence of types possessing delayed germination (0% class in Table II) is satisfactorily accounted for by the assumption that the F_2 recessive embryos, carried by the seeds of the F_1 plants, failed to germinate, thus eliminating non-germinable types from the population of F_2 plants.

The correlation between high degree of germinability and *sativa* (w) type of grain is very marked. There also appears to be a tendency for *fatua* (W) types to be less germinable than intermediate (I) types. The correlation between germinability and grain type, calculated by the coefficient of correlation method, is as follows: $r = .307 \pm .037$ for Cross 8, $r = .385 \pm .062$ for Cross 9, and $r = .086 \pm .071$ for Cross 10.

The nature of the genetic linkage relationship between germinability and grain type may be deduced very simply. It is known definitely that type of grain is inherited on the basis of a single gene or a single gene complex (1). Thus, a linkage relationship with grain type can concern only a single chromosome. Therefore, it may be assumed that one genetic factor for germinability (AA, Aa, or aa) is linked with the factor for grain type (WW, Ww, or ww) in the following manner: $(Wa)(Wa)$, giving the *fatua* type; $(Wa)(wA)$, giving the intermediate type; and $(wA)(wA)$, giving the *sativa* type. It is obvious that there must be at least one factor for germinability in addition to the linked factor, for otherwise all *fatua* types would be non-germinable*.

In the genetic analyses, the observed F_2 data were compared with a series of frequencies calculated on the basis of several theoretical ratios derived from different assumptions regarding numbers and germinative capacities of factors. Alternative assumptions were made regarding the theoretical germinative capacities of genotypes consisting of one heterozygous and a number of recessive factors for germinability ($A_1a_1a_2a_2$ or $a_1a_1A_2a_2$ in the case of two factors; $A_1a_1a_2a_2a_3a_3$ or $a_1a_1A_2a_2a_3a_3$ or $a_1a_1a_2a_2A_3a_3$, in the case of three factors, etc.)**. The single heterozygous factor (Aa, for short) was assumed to be germinable in certain cases and to be non-germinable in others, the alternative assumptions giving markedly different theoretical ratios. An example of the method of calculating these ratios is provided by Table VIII. The theoretical ratios derived from the different assumptions are given in Table III.

The various phenotypic classes are designated by the grain-type symbols (w, I, or W) followed by a number expressing the theoretical germination percentage to the nearest whole number.

It might be useful to illustrate the method used in deriving the phenotypes given in Table III. For example, in the case of the I89 ($A_1a_1A_2a_2A_3a_3$) type of F_2 plant in Assumption 4, the theoretical germinability of the F_3 embryos is 57 germinable (*i.e.*, 16 Aw, 30AI, 11AW) and seven non-germinable (*i.e.*, 2aI, 5aW) which gives an average of 89.10% germinability. (Refer to Table VIII.) Thus, in formulating the phenotypes, the grain type of each F_2 plant was associated with the average theoretical percentage germination of the F_3 embryos borne by the seeds of the plant in question.

To facilitate the comparison of the various theoretical ratios given in Table III with the observed data given in Table II, theoretical frequencies have been calculated for Crosses 8 and 9 and are presented in Table IV. The frequencies for Cross 10 were not calculated because the population of this cross, (88), is practically the same as that of Cross 9, (86).

**The terms germinable and non-germinable are used in this paper to refer, respectively, to ability and inability of seeds to germinate under the conditions of the test in question. Germinability is used as a general term referring to germinative capacities, whether positive or negative. These terms make unnecessary the use of such unwieldy expressions as delayed germination, immediate germination, etc.*

***Henceforth in this paper the condition of a single heterozygous factor and a series of homozygous recessive factors for germinability will be expressed by the symbol Aa.*

TABLE III

THEORETICAL F_2 RATIOS BASED ON VARIOUS ASSUMED NUMBERS AND GERMINATIVE CAPACITIES OF FACTORS

Assumption no.	No. of factor pairs	Genotype of *A. fatua*	Genotype of *A. sativa*	Germinative capacity of *Aa*	Calculated ratios based on seeds of F_1 plants
1	2	$(Wa_1)(Wa_1)a_2a_2$	$(wA_1)(wA_1)A_2A_2$	germinable	4(w100) : 2(I100) : 1(W100) : 4(I94) : 2(I75) : 2(W75) : 1(W0)
2	2	$(Wa_1)(Wa_1)a_2a_2$	$(wA_1)(wA_1)A_2A_2$	non-germinable	4(w100) : 2(I100) : 1(W100) : 4(I69) : 2(I25) : 2(W25) : 1(W0)
3	3	$(Wa_1)(Wa_1)a_2a_2a_3a_3$	$(wA_1)(wA_1)A_2A_2A_3A_3$	germinable	16(w100) : 14(I100) : 7(W100) : 8(I98) : 8(I94) : 4(W94) : 2(I75) : 4(W75) : 1(W0)
4	3	$(Wa_1)(Wa_1)a_2a_2a_3a_3$	$(wA_1)(wA_1)A_2A_2A_3A_3$	non-germinable	16(w100) : 14(I100) : 7(W100) : 8(I89) : 8(I69) : 4(W69) : 2(I25) : 4(W25) : 1(W0)
5	4	$(Wa_1)(Wa_1)a_2a_2a_3a_3a_4a_4$	$(wA_1)(wA_1)A_2A_2A_3A_3A_4A_4$	germinable	64(w100) : 74(I100) : 37(W100) : 16(I99) : 24(I98) : 8(W98) : 12(I94) : 12(W94) : 2(I75) : 6(W75) : 1(W0)
6	4	$(Wa_1)(Wa_1)a_2a_2a_3a_3a_4a_4$	$(wA_1)(wA_1)A_2A_2A_3A_3A_4A_4$	non-germinable	64(w100) : 74(I100) : 37(W100) : 16(I97) : 24(I89) : 8(W89) : 12(I69) : 12(W69) : 2(I25) : 6(W25) : 1(W0)

Comparison of the theoretical frequency distributions of Table IV with the actual data of Table II brings out the following facts:

(1) Assumptions 1, 3, and 5 give theoretical segregation for a non-germinable type and several types with germinability ranging from 75 to 100%. The observed data deviate greatly from such segregation in that numerous well-distributed individuals occur in the range between the zero and 75% classes. Assumptions 1, 3, and 5 are ruled out, therefore, on the basis of theoretical frequency distributions which are in marked disagreement with the observed data.

(2) Assumption 2 may be eliminated from further consideration on the basis of greatly excessive theoretical frequencies in the case of I25 and W25 types.

(3) Assumption 6 provides theoretical frequencies which agree much more closely with the observed data than those of any of the assumptions discussed above. However, in the I 89-100 and W 89-100 classes the theoretical are considerably greater than the observed frequencies, and *vice versa* in the case

TABLE IV

THEORETICAL F_2 FREQUENCIES FOR CROSSES 8 AND 9 CALCULATED ON THE BASIS OF ASSUMPTIONS GIVEN IN TABLE III

Number of cross	Assumption no.	Theoretical frequencies of various phenotypic classes											
		W						I					w
		0	25	69	75	89	94–100	25	69	75	89	94–100	100
8(N=143)	1	8.9	—	—	17.9	—	8.9	—	—	17.9	—	53.7	35.8
	2	8.9	17.9	—	—	—	8.9	17.9	35.8	—	—	17.9	35.8
	3	2.2	—	—	8.9	—	24.5	—	—	4.5	—	67.1	35.8
	4	2.2	8.9	8.9	—	—	15.6	4.5	17.9	—	17.9	31.3	35.8
	5	0.6	—	—	3.4	—	31.8	—	—	1.1	—	70.4	35.8
	6	0.6	3.4	6.7	—	4.5	20.7	1.1	6.7	—	13.4	50.3	35.8
9(N=86)	1	5.4	—	—	10.8	—	5.4	—	—	10.8	—	32.3	21.5
	2	5.4	10.8	—	—	—	5.4	10.8	21.5	—	—	10.8	21.5
	3	1.3	—	—	5.4	—	14.8	—	—	2.7	—	40.4	21.5
	4	1.3	5.4	5.4	—	—	9.4	2.7	10.8	—	10.8	18.8	21.5
	5	0.3	—	—	2.0	—	19.1	—	—	0.7	—	42.3	21.5
	6	0.3	2.0	4.0	—	2.7	12.4	0.7	4.0	—	8.0	30.2	21.5

of the I 69, W 69, I 25 and W 25 classes. The theoretical ratios of assumption 6 are considered, however, to show a sufficiently close agreement with the observed data to warrant further consideration in goodness-of-fit tests.

(4) Assumption 4 is found to provide theoretical frequencies which are on the whole in good agreement with the observed data, though this agreement is somewhat obscured by the straggling distribution of intermediate classes. The agreement is much better than that of any of the other assumptions discussed. The only considerable deviations between theoretical and observed frequencies occur in the case of W25 types, where the theoretical are much too high in Crosses 8 and 10. These deviations and the straggling nature of the intermediate classes demand special consideration.

It would appear from the above discussion that there is at least a fair possibility of Assumption 4 providing a basis for an explanation of the inheritance of germinability; therefore, the relations between this assumption and the observed data will be discussed in considerable detail.

A point to be settled before taking up this discussion involves the decision of whether the non-germinability of seeds of F_1 plants (see Table I) should be considered as genetic or environmental in nature. A number of facts strongly indicate that non-germinability was for the most part, if not entirely, due to the genetic nature of the seeds in question. In the first place, the seeds were carefully selected for strong vitality (see section on Materials and Methods) and germinative conditions were very favorable. Secondly, 100% germination occurred in the *A. sativa* parental check. And thirdly, a series of several other *A. sativa* varieties (checks in tests for delayed germination in fatuoids), which had been grown in the greenhouse with the F_1 plants, showed practically 100% germination in tests which were directly comparable to those of the seeds of F_1 plants. It is reasoned that the non-germination of seeds of F_1 plants, with possibly one or two exceptions, must be considered as genetic expressions.

Assumption 4 implies that the single heterozygous factor *Aa* is potentially non-germinable. This being the case, the I 25 and W 25 classes should have been eliminated with the W0 types when the seeds of the F_1 plants were tested. Such eliminations occurred to a large extent, apparently, in the case of W 25 types in Crosses 8 and 10; but not in the case of I 25 types in any of the crosses, nor of W 25 types in Cross 9 (Compare Tables II and IV). It will be noted by reference to Table I that excessive numbers of non-germinated seeds occurred in the case of the F_1 plants of Crosses 8 and 10; further, it may be noted that the degree to which these numbers are in excess of theoretical W0 frequencies corresponds to the degree to which observed W 25 numbers are deficient relative to theoretical frequencies (Compare Tables I, II, and IV). It is significant to note in this connection that in Cross 9, where the W 25 observed corresponds closely to the theoretical frequency, the observed number of non-germinable seeds of the F_1 plant is also in close agreement with the theoretical W0 frequency.

A possible explanation of the fact that F_2 embryos with *Aa* genotypes germinated in some and not in other cases (see Table I) would be to assume that, at the time of testing the seeds of F_1 plants, these embryos were passing through the critical stage in the after-ripening process where some were barely germinable while others had not quite after-ripened sufficiently to germinate. It is also possible that these variations in germinability may be explained on the basis of different germinative potentialities of the different factors.

It will also be necessary to explain why, in Crosses 8 and 10, W 25 types frequently failed to germinate while I 25 types appeared to germinate in nearly all cases. The explanation lies, in all probability, in the fact that different factors are involved in producing the two types. W 25 types are always produced by a single non-linked heterozygous factor (A_2a_2 or A_3a_3), while I 25 types are always produced by the heterozygous condition of the linked factor (A_1a_1). (See Table VIII.) This explanation assumes that the linked factor is more potent than the non-linked factor, and, therefore, produces the germinable state after a shorter period of after-ripening.

Theoretical frequencies calculated on the basis of Assumptions 4 and 6, respectively, have been tested for goodness-of-fit to the observed data by the method described by Hayes and Garber (4). These tests are summarized in Tables V and VI. With respect to Crosses 8 and 10, the observed frequencies of W 25 classes in each cross and the frequencies of non-germinated seeds of the respective F_1 plants have been grouped into a single class considered to represent the combined frequencies of W0 and W 25 classes for the cross in question. In the case of Cross 9 no groupings were made, the single non-germinable seed of the F_1 plant being considered as the W0 frequency.

The dividing lines in Table II indicate the manner in which the W 25 and I 25 classes were divided from W 69 and I 69 classes, and also how the latter were separated from the W 89-100 and I 89-100 classes. The deviation in the line separating the higher classes, is made in consequence of the additional phenotypes and the greater frequencies in the I 89-100 classes.

In Table V, where the theoretical frequencies are based upon Assumption 4, the goodness-of-fit tests indicate very close correspondence between these frequencies and the observed data in all crosses. The values of P are as follows: between .90 and .95 for Cross 8, between .8 and .9 for Cross 9, and between .5 and .7 for Cross 10. The values of P were determined from a table provided by Fisher (2).

TABLE V

OBSERVED F_2 DATA (INCLUDING NON-GERMINABLE TYPES) OF CROSSES 8, 9 AND 10 COMPARED WITH THEORETICAL FREQUENCIES CALCULATED ON THE BASIS OF ASSUMPTION 4. VALUES OF χ^2 AND P FOR EACH CROSS ARE ALSO GIVEN.

Phenotypic class	Cross 8		Cross 9		Cross 10	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
w100	39	37.5	23	21.8	22	23.0
W100	19	16.4	12	9.5	15	10.1
I100 I89	50	51.6	32	29.9	33	31.6
I69	16	18.8	8	10.9	10	11.5
W69	12	9.4	6	5.4	6	5.8
I25	4	4.7	1	2.7	2	2.9
W25 W0	10	11.7	4 1	5.4 1.4	4	7.2
N	150	150.1	87	87.0	92	92.1
χ^2	2.0092		3.2575		4.3868	
P	between .90 and .95		between .80 and .90		between .50 and .70	

In Table VI, where the theoretical frequencies are based upon Assumption 6, the goodness-of-fit tests indicate a very poor correspondence between these

TABLE VI

OBSERVED F_2 DATA (INCLUDING NON-GERMINABLE TYPES) OF CROSSES 8, 9 AND 10 COMPARED WITH THEORETICAL FREQUENCIES CALCULATED ON THE BASIS OF ASSUMPTION 6. VALUES OF χ^2 AND P FOR EACH CROSS ARE ALSO GIVEN

Phenotypic class	Cross 8		Cross 9		Cross 10	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
w100	39	37.5	23	21.8	22	23.0
W100 W89	19	26.4	12	15.3	15	16.2
I100 I97 I89	50	66.8	32	38.8	33	41.0
I69	16	7.0	8	4.1	10	4.3
W69	12	7.0	6	4.1	6	4.3
I25	4	1.2	1	0.7	2	0.7
W25 W0	10	4.1	4 1	2.0 0.3	4	2.2
N	150	150.0	87	87.1	92	91.7
χ^2	36.5256		10.3219		13.8083	
P	<.01		between .10 and .20		between .02 and .05	

frequencies and the observed data of all three crosses. The values of P are as follows: less than .01 for Cross 8, between .1 and .2 for Cross 9, and between .02 and .05 for Cross 10.

The results of these goodness-of-fit tests serve to eliminate Assumption 6 from further consideration, and to establish Assumption 4 as the basis of a tentative explanation of inheritance in the F_2.

The question of the possibility of genetic explanations other than those already studied should receive some attention. Studies revealed that as the number of factors was increased above three, the frequencies of the highly germinative phenotypic classes increased at the expense of the frequencies of the lower germinative classes, rendering theoretical frequencies more and more unlike the observed data. Nor was it possible to justify, on the basis of observed data, assumptions regarding germinative capacities of factors such as, for example, the non-germinability of *AA* and *AaAa* types*. After a thorough examination of alternative explanations, it is concluded that Assumption 4 provides, as far as can be determined, the only possible basis of explaining the observed data.

The significance of the P values calculated in Table V should be examined from the point of view of the justifiability of the divisions of phenotypic classes made in Table II. The positions of the dividing lines in Table II are based upon natural, though not always very distinct, divisions in the frequency distribution. The line dividing the W 25 and I 25 classes from the W 69 and I 69 classes runs through a natural break in the distribution. The line separating the W 100 class from the W 69 class is determined by the range shown in the w 100 class, which is a natural group, as well as by points of low frequency in the distribution. The line dividing the I 89 and I 100 classes from the I 69 is extended beyond the lines of the w 100 and W 100 classes in order to include the natural range of these combined classes. It will be noted that this line, like all other lines, runs through points of low frequency on either side of which the frequencies increase. It is felt that the natural distribution of the data given in Table II justifies the divisions made between phenotypic classes.

The straggling nature of the distribution of the intermediate classes (I 69 and W 69) should, if possible, be accounted for. In considering this matter it should be borne in mind that the variability in the observed germination percentages may be due to either or both of two things: the occurrence of different degrees of non-germination among types theoretically expected to germinate, and the chance deviations from the theoretical segregation ratios. (Each frequency represents the average germination percentage of a segregating line of F_3 embryos). Variability due to these two causes is sufficient to explain the range of germinability in the intermediate classes of Cross 9, but in the case of Crosses 8 and 10 it appears probable that still another factor influenced variability. It may be that, owing to internal or external

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**Genotypes are simplified by the omission of homozygous recessive factors.*

conditions affecting the material of Crosses 8 and 10 at the time of testing, the *AaAa*. and *AA* types were in some cases non-germinable. Theoretically, the non-germinability of these types would transform I 69 and W 69 classes into I 44 and W 44 classes, respectively. This explanation would also account for the occurrence of two w types of intermediate germinability*. There is also a possibility that differences in the germinative potentialities of the different factors may explain some of these variations in germinability.

The analysis and discussion of F_2 data has been carried to a point where it is possible to state a definite hypothesis in explanation of the inheritance of germinability. The hypothesis is, briefly, as follows: Germinability is inherited as a dominant character on the basis of three factors of essentially equal potency, one of which is linked with the factor for grain type; the embryos having genotypes with a single heterozygous factor for germinability (*Aa*) are potentially non-germinable under the conditions of the tests, but may be germinable in relatively rare instances; the embryos having genotypes with one homozygous (*AA*) or two heterozygous (*AaAa*) factors for germinability are potentially germinable under the conditions of the tests, but may be non-germinable in relatively rare instances; with the exception of the triple recessive all other genotypes are potentially germinable.

The validity of this hypothesis will be examined further in the light of F_3 data.

THE THIRD GENERATION

It has been shown that the seeds of F_3 plants possess F_4 embryos, therefore, the result of a germination test of the seeds of an F_3 plant represents the average germinability of a complete line of F_4 embryos. The averages of germinability of F_4 lines represent the germinative potentialities of the respective F_3 genotypes.

Sixty-six lines of F_3 plants from Cross 8 and 18 from Cross 10 were tested for germinability. These tests were continued over a period extending from two and a half to five months after harvesting, which was within the range between the time when the *A. sativa* parent was completely germinable and the time when the *A. fatua* parent began to show germinability. This period of testing was very similar, with respect to time after harvesting, to the time of the tests of F_2 plants.

The coefficient of correlation for the percentage germination obtained in the F_2 tests and the average percentage of germination of the plants of F_3 lines was .517 ± .061 for Cross 8 and .760 ± .67 for Cross 10.

Environmental variations in germinability resulting from differences in the period of after-ripening given the various F_3 lines prior to testing have made impossible the brief summarization of the F_3 data in tabular form. Consequently, it has been necessary to limit the presentation of the F_3 results to general descriptions of breeding behavior in which the lines from each class of F_2 phenotypes have been considered as a separate group.

**The* w *plant of lowest germinability gave 79% germination when tested in the* F_3.

Table VII was prepared with the view of simplifying the discussion of relations between observed and theoretical frequencies in the F_3 segregation for germinability and grain types. The theoretical F_3 ratios given in this table were derived by averaging the theoretical germination percentages expected in the F_4 (embryonic generation) lines from each F_3 grain type, and then relating these averages directly to the respective F_3 grain types (see Table VIII).

TABLE VII

F_3 RATIOS EXPECTED FROM THE VARIOUS F_2 TYPES ON THE BASIS OF THE HYPOTHESIS. THE NUMBER OF TESTED F_3 LINES FROM EACH F_2 TYPE IS ALSO GIVEN

F_2 type	No. of F_3 lines tested	Theoretical F_3 ratios
w100	16	w100
I100	14	I100
I89	12	16(w100) : 14(I100) : 8(I89) : 8(I69) : 2(I25) : 7(W100) : 4(W69) : 4(W25) : 1(W0)
I69	15	4(w100) : 2(I100) : 4(I69) : 2(I25) : 1(W100) : 2(W25) : 1(W0)
I25	5	1(w100) : 2(I25) : 1(W0)
W100	5	W100
W69	13	7(W100) : 4(W69) : 4(W25) : 1(W0)
W25	4	1(W100) : 2(W25) : 1(W0)
W0	0	W0
Total......	84	

The F_3 data are, on the whole, in good agreement with the theoretical frequencies expected on the basis of the hypothesis. However, many F_3 lines tended to show much lower percentages of germination than would be expected theoretically, the occurrence of which appeared to be correlated with early testing with respect to all grain types but particularly in the case of W types.

Germinability of F_3 lines from w F_2 types varied from 50 to 99.3%. Distinctly low germinability was shown by only three lines, each of which was tested between two and a half and three and a half months after harvesting. The remainder of the lines tested during this period showed reasonably high germinability (up to 94%). These results may be explained by assuming that w types arising from F_2 types having genotypically only two or three dominant allelomorphs for germinability(AA or $AAAa$) were incapable of high germination in early tests, while w types possessing four to six dominant allelomorphs were relatively highly germinable at this time. The average germination of all w lines tested was 84%, while with the three low-germinating lines omitted it was 91%.

Two F_3 lines from I 100 F_2 types showed relatively low average germination percentages, both being among the lines tested in the early part of the testing period. These two lines are presumed to have arisen from F_2 types having genotypically only three dominant allelomorphs ($AaAA$). The average germination of all I 100 F_3 lines tested was 86%, while with the two lines of lower germination omitted it was 90%.

Four out of the five F_3 lines from W 100 F_2 types were tested between three and three and a half months after harvesting and gave germination percentages between 32 and 71 with an average of 49%; the remaining line was tested five months after harvesting and gave 89% germination. These facts indicate that at least one of the non-linked factors is somewhat less potent than the linked factor, thus requiring a longer period of after-ripening to produce the germinable state. (W types never possess either of the dominant allelomorphs of the linked factor).

In most cases the observed data of F_3 lines from I 89 F_2 types were in close agreement with the theoretical frequencies, values of P running as high as .95. In some cases, however, the values of P were as low as .01 owing, for the most part, to very low observed frequencies in the W 100 and W 69 classes and to very high frequencies in the W 25 and W 0 classes. The occurrence of these lines with a lower degree of germination than was expected, appeared to be correlated with early testing. Early testing may be expected to cause *AaAa* and *AA* types to tend toward non-germinability, particularly when these types concern the less potent non-linked factors, upon which W types must depend for germinability. This would tend to shift W 100 and W 69 classes toward the W 25 class and to shift the W 25 class toward the W 0 class. Granting that the observed deviations may be explained in this way, the data of F_3 lines from I 89 F_2 types may be said to be in close correspondence with the theoretical frequencies.

The discussion applied to the F_3 lines from I 89 F_2 types applies essentially to the F_3 lines from I 69 F_2 types. The values of P for the latter lines range from .8 to .05.

The observed data of the F_3 lines from I 25 F_2 types showed very close correspondence with the theoretical ratio. Only one of these lines was tested less than four months after harvesting. This line, tested three months after harvesting, showed a tendency toward low germinability in both w and I segregates; early testing, of course, could not reduce the germinability of the W segregates, they being of the W 0 type.

In the case of F_3 lines from W 69 F_2 types, six showed good correspondence between observed and theoretical frequencies, while seven showed a deficiency of observed frequencies in the W 100 and W 69 classes with an excess in the W 25 and W 0 classes. The occurrence of lines with lower degrees of germination than the expected was correlated with early testing. An explanation of these deviations from theoretical frequencies has been given in connection with the discussion of lines from I 89 F_2 types.

The F_3 lines from W 25 F_2 types were all tested less than three and a half months after harvesting, and all show a high deficiency of observed frequencies in the W 100 class and a great excess of frequencies in the W 0 class. These deviations from theoretical expectations may be explained on the basis of the non-germinability of *AA* types due to premature testing.

In no case did the F_3 results make it necessary to change the classification of an F_2 plant. However, in certain instances the restricted germination which occurred in certain early-tested lines made it impossible definitely to associate F_3 segregation with F_2 types.

It would appear, judging from the tendency toward low germinability observed in F_3 lines tested about three months after harvesting, that a longer period of after-ripening was required to render *AA* and *AaAa* types germinable in F_3 plants grown in Washington than was required in F_2 plants grown in Alberta; for, in the case of F_2 plants of Cross 9, tested three months after harvesting, there was little indication of non-germinability of *AA* and *AaAa* types.

A further test of the hypothesis was made by marking in a number of F_3 rows the point in the row up to which the seedlings from readily germinable F_2 seeds were transplanted, and later observing the grain type of the plants on either side of the mark. F_3 lines from four I 25 and six I 69 F_2 types were studied in this manner.

In a line from an I 25-type F_2 plant which gave very nearly 25% germination, the plants from readily germinable seeds were practically all of the w type, while the plants from artificially germinated seeds were all of the I and W types This indicates that *AA* genotypes were germinable while *Aa* and *aa* were non-germinable. However, in a line from an F_2 plant which gave considerably more than 25% germination, I- as well as w-type plants were produced from readily germinable seeds, indicating that the high germination percentage was at least partly due to the germination of a number of *Aa* type embryos. Conversely, in a line from an F_2 plant which gave far less than 25% germination several w plants were produced from artificially germinable seeds, indicating that the low germination percentage was in this case at least partly due to the non-germination of a number of *AA* type embryos.

In F_3 lines from I 69 F_2 types it was found that readily germinable seeds produced w, I and W plants in about the expected proportion (4w : 6I : 1W), and the plants from artificially stimulated seeds also occurred in about the expected proportion (2I : 3W).

These observations on the grain type of plants produced from naturally and artificially germinated seeds, which, however, represent at best only a partial elucidation of F_3 segregation, afforded in every case excellent support to the hypothesis.

The coefficients of correlation for grain type and percentage germination in F_3 lines from I-type F_2 plants ranged from .000 ± .114 in the case of certain I 100 types to .904 ± .21 in the case of certain I 25 types.

Granting certain assumptions which are believed to be justified, none of the experimental results of the F_3 can be cited as definite evidence against the validity of the hypothesis based on the F_2; but, on the other hand, many data afford support to the hypothesis. The F_3 results showed even more clearly than the F_2 that one factor for germinability is linked with the factor for grain type, and they supported the assumption that there are two non-

TABLE VIII

INHERITANCE SCHEME BASED UPON ASSUMPTION 4 (SEE TABLE III)

$$(wA_1)(wA_1)A_2A_2A_3A_3 \times (Wa_1)(Wa_1)a_2a_2a_3a_3$$

(*A. sativa*) (*A. fatua*)

F_1 $(wA_1)(Wa_1)A_2a_2A_3a_3$

	F_2 genotypes	F_2 phenotypes*	F_3 phenotypes	F_3 Av. % germ.	F_4 phenotypes	F_4 Av. % germ.
1	$(wA_1)(wA_1)\ A_2A_2A_3A_3$	Aw	Aw	100	Aw	100
2	$(wA_1)(wA_1)\ A_2A_2A_3a_3$	Aw	Aw	100	Aw	100
2	$(wA_1)(wA_1)\ A_2a_2A_3A_3$	Aw	Aw	100	Aw	100
2	$(wA_1)(Wa_1)\ A_2A_2A_3A_3$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1Aw : 2AI : 1AW) : 1(AW)	100
4	$(wA_1)(wA_1)\ A_2a_2A_3a_3$	Aw	Aw	100	Aw	100
4	$(wA_1)(Wa_1)\ A_2A_2A_3a_3$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1AW : 2AI : 1AW) : 1(AW)	100
4	$(wA_1)(Wa_1)\ A_2a_2A_3A_3$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1AW : 2AI : 1AW) : 1(AW)	100
8	$(wA_1)(Wa_1)\ A_2a_2A_3a_3$	AI	16Aw : 30AI : 2aI : 11AW : 5aW	89.10	16(Aw) : 14(1Aw : 2AI : 1AW) : 2(1Aw : 2aI : 1aW) : 8(16Aw : 30AI : 2aI : 11AW : 5aW) : 7(AW) : 1(aW) : 8(4Aw : 6AI : 2aI : 1AW : 3aW) : 4(11AW : 5aW) : 4(3aW : 1AW)	84.18
1	$(wA_1)(wA_1)\ A_2A_2a_3a_3$	Aw	Aw	100	Aw	100
2	$(wA_1)(wA_1)\ A_2a_2a_3a_3$	Aw	Aw	100	Aw	100
2	$(wA_1)(Wa_1)\ A_2A_2a_3a_3$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1Aw : 2AI : 1AW) : 1(AW)	100
4	$(wA_1)(Wa_1)\ A_2a_2a_3a_3$	AI	4Aw : 6AI : 2aI : 1AW : 3aW	68.75	4(Aw) : 2(1Aw : 2AI : 1AW) : 2(1Aw : 2aI : 1aW) : 4(4Aw : 6AI : 2aI : 1AW : 3aW) : 1(AW) : 2(3aW : 1AW) : 1(aW)	67.19
1	$(wA_1)(wA_1)\ a_2a_2A_3A_3$	Aw	Aw	100	Aw	100
2	$(wA_1)(wA_1)\ a_2a_2A_3a_3$	Aw	Aw	100	Aw	100
2	$(wA_1)(Wa_1)\ a_2a_2A_3A_3$	AI	1Aw : 2AI : 1AW	100	1(Aw) : 2(1Aw : 2AI : 1AW) : 1(AW)	100
4	$(wA_1)(Wa_1)\ a_2a_2A_3a_3$	AI	4Aw : 6AI : 2aI : 1AW : 3aW	68.75	4(Aw) : 2(1Aw : 2AI : 1AW) : 2(1Aw : 2aI : 1aW) : 4(4Aw : 6AI : 2aI : 1AW : 3aW) : 1(AW) : 2(3aW : 1AW) : 1(aW)	67.19
1	$(Wa_1)(Wa_1)\ A_2A_2A_3A_3$	AW	AW	100	AW	100
2	$(Wa_1)(Wa_1)\ A_2A_2A_3a_3$	AW	AW	100	AW	100
2	$(Wa_1)(Wa_1)\ A_2a_2A_3A_3$	AW	AW	100	AW	100
4	$(Wa_1)(Wa_1)\ A_2a_2A_3a_3$	AW	11AW : 5aW	68.75	7(AW) : 4(11AW : 5aW) : 4(3aW : 1AW) : 1(aW)	67.19

TABLE VIII—*Concluded*

INHERITANCE SCHEME BASED UPON ASSUMPTION 4 (SEE TABLE III)

$(wA_1)(wA_1)A_2A_2A_3A_3 \times (Wa_1)(Wa_1)a_2a_2a_3a_3$
(*A. sativa*) (*A. fatua*)

F_1 $(wA_1)(Wa_1)A_2a_2A_3a_3$

	F_2 genotypes	F_2 phenotypes	F_3 phenotypes	F_3 Av. % germ.	F_4 phenotypes	F_4 Av. % germ.
1	$(wA_1)(wA_1)\ a_2a_2a_3a_3$	Aw	Aw	100	Aw	100
2	$(wA_1)(Wa_1)\ a_2a_2a_3a_3$	AI	1Aw : 2aI : 1aW	25.00	1(Aw) : 2(1Aw : 2aI : 1aW) : 1(aW)	37.50
1	$(Wa_1)(Wa_1)\ A_2A_2a_3a_3$	AW	AW	100	AW	100
2	$(Wa_1)(Wa_1)\ A_2a_2a_3a_3$	aW	3aW : 1AW	25.00	1(AW) : 2(1AW : 3aW) : 1(aW)	37.50
1	$(Wa_1)(Wa_1)\ a_2a_2A_3A_3$	AW	AW	100	AW	100
2	$(Wa_1)(Wa_1)\ a_2a_2A_3a_3$	aW	3aW : 1AW	25.00	1(AW) : 2(1AW : 3aW) : 1(aW)	37.50
1	$(Wa_1)(Wa_1)\ a_2a_2a_3a_3$	aW	aW	0.00	aW	0.00

F_2 ratio: 16(w100) : 14(I100) : 7(W100) : 8(I89) : 8(I69) : 4(W69) : 2(I25) : 4(W25) : 1(W0) linked factors for germinability. It was shown in the F_3 that the balance between germinability and non-germinability in embryos with *Aa*, *AA*, or *AaAa* genotypes is somewhat unstable and may be influenced by relatively small variations in the state of after-ripening, a fact which lends justification to certain assumptions made in explaining irregularities in F_2 results. The F_3 results also served to establish on a firmer basis the probability, suggested somewhat vaguely by the F_2, that the linked factor possesses more potent germinative capacities than do the non-linked factors.

**In phenotypic symbols,* A *and* a *denote germinability and non-germinability, respectively;* w, I, *and* W *denote sativa, intermediate and fatua grain types, respectively.*

However, the occurrence of many deviations from the theoretically expected results, while perhaps satisfactorily explained, makes it impossible to lay claim to the proving of the hypothesis. It is concluded that F_3 results, while not affording definite proof, strongly indicate that the hypothesis, in its essential features, represents the true explanation of the inheritance of germinability.

General Discussion and Conclusions

The results from the first three hybrid generations of plants have been analyzed and in most respects more or less fully discussed. It now remains to discuss, briefly, certain matters bearing on the analysis as a whole and to draw conclusions regarding the essential nature of the inheritance of germinability.

The data of the second and third generations have been in a general way characterized by a lack of clear-cut distinctions between phenotypic classes, and by a frequent occurrence of irregularities in germinative breeding results. This state of affairs made genetic analysis more difficult and probably less convincing than would have been the case under more favorable circumstances.

The validity of the whole genetic analysis hinges upon the acceptability of the following assumption: that under the conditions of nearly all tests, embryos with *Aa* genotypes were non-germinable, but that in a very few tests they were germinable; and, that under the conditions of most tests embryos with *AA* or *AaAa* genotypes were germinable, but that in a few cases they were non-germinable. This assumption, if granted, would account for the straggling nature of the F_2 and F_3 frequency distributions and for the observed irregularities in breeding results; it would place the observed frequencies in harmony with the theoretical frequencies, and thus serve in establishing the hypothesis.

A number of results afforded definite support to this assumption: (1) The observations on the grain type of plants grown from seeds which germinated naturally and from seeds in which germination was artificially stimulated indicated that embryos with *Aa* and *AA* genotypes could be either germinable. or non-germinable in the same test. (2) It was found in F_3 tests that the type of germinability in which the non-germinability of embryos with *Aa*, *AA* or *AaAa* genotypes is assumed was correlated with early testing. It is inferred that very shortly after harvesting only embryos with six dominant allelomorphs (*AAAAAA*) are germinable, and that as time goes on embryos with a progressively smaller number of dominant allelomorphs become germinable

Thus, at a certain stage embryos with *Aa*, *AA*, *AaAa* or triple recessive genotypes will be non-germinable; later, *AA* and *AaAa* types will be germinable; still later the *Aa* types will germinate; and, finally, the triple recessive (*A. fatua*) types will germinate. Of course, it is to be expected that there would be considerable overlapping of the periods when germinability occurs in the successive types. This line of reasoning is in accord with the assumption under discussion.

Evidence was provided by the F_3 which indicated that at least one of the non-linked factors possessed less potent germinative capacities than the linked factor, and thus was capable of producing germinability only after a longer period of after-ripening than was required by the linked factor. However, this difference in potency is not considered great enough to receive special consideration in the general statement of inheritance.

Crossing over has not been discussed because of the fact that the data are not suitable for its study; the presence of two non-linked factors for germinability would, in most cases, obscure its expression; and, in instances where the non-linked factors are in a recessive condition, the variation in germinability of A_1A_1 and A_1a_1 genotypes would make it difficult or impossible to establish its occurrence.

Since the F_3 results have not altered the essential nature of the hypothesis based on the F_2, which has been adequately discussed, it is believed unnecessary to carry the general discussion further.

It is concluded that the experimental results, as analyzed, give very strong indication that the mode of inheritance of germinability in the hybrids of *A. fatua* and *A. sativa* is, in its essential features, as follows: Germinability is dominant over non-germinability (delayed germination) and is inherited on the basis of three factors of more or less similar germinative potencies, one of which is linked with the factor for grain type. Under the conditions of the tests, embryos with *Aa* genotypes were in nearly all cases non-germinable but in certain relatively rare cases were germinable; embryos with *AA* or *AaAa* genotypes were to a very great extent germinable but in a few instances were non-germinable; embryos with recessive genotypes were non-germinable; embryos having genotypes with three or more dominant allelomorphs were germinable.

This mode of inheritance presumes the following parental genotypes:

A. sativa, $(wA_1)(wA_1)A_2A_2A_3A_3$
A. fatua, $(Wa_1)(Wa_1)a_2a_2a_3a_3$

Assuming these genotypes, the F_2 ratio from the cross between these two species is approximately, 16(w100) : 14(I100) : 7(W100) : 8(I89) : 8(I69) : 4(W69) : 2(I25) : 4(W25) : 1(W0).

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CHARACTER COMBINATIONS IN RELATION TO ENDOSPERM DEVELOPMENT IN INTER-SPECIFIC *TRITICUM* CROSSES[1]

BY J. B. HARRINGTON[2]

Abstract

A study was made of the frequency of different character combinations in relation to seed plumpness in the F_2 generation of crosses of *T. durum*, *T. dicoccum* and *T. persicum* with *T. vulgare*. Some inter-specific character combinations occurred frequently and others were rare, which was expected since the characters varied markedly in their frequency of inter-specific combinations with other characters. The data did not support the popular assumption that inter-specific character combinations of value were to be found largely in plants from shrunken seeds rather than in those from plump seeds. Plants from plump seeds possessed nearly as large a proportion of the combinations which were missing in the plants from shrunken seeds as did the latter plants with respect to the former. The difference could not be considered significant and none of the missing combinations appeared to be important as far as practical breeding is concerned.

Introduction

Marked success has been achieved in producing desirable character combinations from crosses between wheats of different chromosome numbers. Examples of this success are found in the work of Hayes *et al.* (4), McFadden (5), Harrington (1), A. A. Sapehin (7) and L. A. Sapehin (8).

In recent studies made by Harrington (2) and Harrington and Marshall (3) on crosses between *vulgare* and three 14-chromosome species, it was demonstrated that a definite relation existed between the degree of development attained by the F_2 seed endosperm and various morphologic characters of the F_2 plant. In general, the most shrunken seeds produced the most *vulgare*-like plants although many shrunken seeds did not produce *vulgare*-like plants and many plump seeds did not produce plants resembling the 14-chromosome parental variety. Desirable *vulgare*-like plants were obtainable from each plumpness class, the largest proportion being from the shrunken seeds. In the material studied, certain character combinations were relatively frequent, others were infrequent and some did not appear at all. It was apparent that the differences were not all casual and therefore that some combinations might be rarely or perhaps never obtained.

A study was made, therefore, of the frequency of different character combinations in relation to seed plumpness for each of the crosses, Iumillo (*durum*) × Marquis (*vulgare*), Vernal (*dicoccum*) × Marquis, and Marquis × Black Persian (*persicum*). The present paper gives the results of this study. The description of parents, description of hybrids and general analysis of the relations between seed plumpness and various morphological characters are presented fully in the paper by Harrington and Marshall (3).

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Contribution from the laboratories of the University of Saskatchewan, Canada, with financial assistance from the National Research Council of Canada. This study forms a part of a co-operative attack on the problem of cereal rust in Canada, carried on jointly by the National Research Council, the Federal Department of Agriculture and the Universities of Manitoba, Saskatchewan and Alberta. The results were reported in full at the Meeting of the Associate Committee on Field Crop Diseases at Winnipeg on April 4, 1935.

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It was shown in the paper by Harrington and Marshall (3) that the *vulgare*-like plants from plump seeds were more *vulgare*-like than the *vulgare*-like plants from shrunken seeds in the IM cross (Iumillo, (*T. durum*) × Marquis, (*T. vulgare*)) but the opposite was true in the VM cross (Vernal, (*T. dicoccum*) × Marquis, (*T. vulgare*)) and the MP cross (Marquis, (*T. vulgare*) × Black Persian, (*T. persicum*)) gave results resembling those of the VM cross. The question then arose, were *all* of the *vulgare*-like plants from shrunken seeds of the IM cross less *vulgare*-like than those from plump seeds, and, if not, were they consistently unlike *vulgare* in certain characters? Similarly it was desirable to ascertain whether the *vulgare*-like plants from plump seeds of the VM and MP crosses were consistently unlike the *vulgare*-like plants from shrunken seeds in certain characters.

TABLE I

DESCRIPTION OF THE *vulgare*-LIKE PLANTS FROM PLUMP F_2 SEED OF THE THREE CROSSES

Cross	Plant number	Spike form	Spike compactness	Stem hollowness	Stem thickness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Beak character	Keel prominence	Brush character	Seed character	Rachis articulation	Awning	Glume adherence
IM	4	I	V	IV	V	V	V	IV	I	IV	*ID*	I	V	IV			
	12	V	I	V	V	V	V	V	V	V	V	I	V	V			
	13	V	V	I	V	I	V	V	IV	I	*D*	I	V	V			
	15	*D*	*D*	IV	IV	V	*D*	V	I	IV	V	IV	V	V			
	16	IV	V	IV	V	V	V	V	V	*D*	V	*ID*	I	IV			
	17	I	V	IV	V	V	V	V	V	*ID*	V	*ID*	IV	*ID*			
	22	V	V	IV	I	IV	V	V	V	V	IV	I	V	IV			
	27	IV	V	V	V	V	V	V	V	V	V	IV	V	V			
	29	IV	V	IV	I	V	V	V	V	I	*ID*	I	V	IV			
	37	I	IV	*ID*	I	V	V	V	IV	*ID*	*D*	IV	V	IV			
	44	I	I	IV	*D*	IV	V	V	V	V	V	V	V	IV			
	49	I	V	IV	V	V	IV	V	*ID*	V	V	I	IV	*ID*			
	51	V	V	V	V	IV	V	V	V	V	V	IV	V	IV			
	58	V	V	V	V	V	IV	V	V	V	V	V	V	*ID*			
	62	I	IV	I	*ID*	I	*D*	V	V	IV	V	IV	V	V			
	71	V	IV	V	I	V	V	V	V	V	V	V	V	V			
	72	IV	V	V	V	V	V	V	V	V	V	V	V	IV			
	73	IV	V	I	I	IV	V	V	V	V	V	V	I	V			
	74	V	V	I	IV	V	V	IV	I	I	*D*	IV	I	*ID*			
	83	*ID*	IV	V	IV	V	V	V	V	V	I	IV	V	IV			
	85	*D*	V	I	I	IV	V	V	V	V	D	IV	V	*ID*			
MP	2			V	*D*	V	V	*ID*	V		*ID*						
	5			IV	*ID*	V	V	*D*	IV		I						
	24			I	V	IV	V	I	*ID*		*ID*						
	35			IV	I	IV	V	V	V		V						
	47			IV	I	V	V	IV	*D*		V						
VM	3	V	V	I	*ID*	IV	V	*D*	*ID*	IV					V	*D*	V
	9	I	V	I	IV	I	IV	I	*ID*	V					*D*	V	I
	17	*ID*	*D*	IV	IV	V	I	IV	IV	*ID*					*D*	IV	*D*
	45	IV	V	IV	IV	V	I	*D*	*D*	V					V	*D*	V

NOTE:—V = *resembling vulgare closely;* D = *resembling 14-chromosome species closely;* I = *intermediate between* V *and* D; IV = *intermediate between* V *and* I; ID = *intermediate between* D *and* I. *Character resemblances to the 14-chromosome species are italized.*

In order to answer these questions the character of each of the *vulgare*-like plants from plump seeds was examined and compared with the character of *vulgare*-like plants from shrunken seeds. In Table I, a character description of each *vulgare*-like plant from plump seeds is given and wherever a plant shows resemblance to the 14-chromosome species as indicated by D or ID the letters are italicized.

In none of the crosses does the distribution of D and ID appear to be strictly random. This is in accordance with expectation since for some characters, notably spike form, spike compactness, rachis hairiness and shoulder width, the condition expressed in the 14-chromosome variety is partially dominant and therefore shows throughout the entire F_2 population many more D and ID than V and IV. Conversely, one character showed dominance of the condition expressed in the *vulgare* variety, Marquis. Where the distribution for a character reveals no dominance, it would be expected that at least one out of a score of *vulgare*-like plants would be D or ID for that character. In the IM cross no D or ID condition was found in any of the 21 plants for the characters, rachis width, shoulder width and brush character. None of the five *vulgare*-like plants of the MP cross nor of the four *vulgare*-like VM plants were D or ID for rachis width, rachis hairiness, or stem hollowness. The fact that all three crosses lack D and ID for rachis width appears to be significant notwithstanding the small numbers of plants concerned.

In Table II the description of the *vulgare*-like plants from shrunken seeds of each cross is given for the characters not represented by D or ID in the

TABLE II

DESCRIPTION OF *vulgare*-LIKE PLANTS FROM SHRUNKEN SEEDS FOR CHARACTERS NOT REPRESENTED BY D OR ID IN THE *vulgare*-LIKE PLANTS FROM PLUMP SEEDS

Cross	Plant No.	Rachis width	Shoulder width	Brush characters	Cross	Plant No.	Stem hollowness	Rachis width	Rachis hairiness	Cross	Plant No.	Stem hollowness	Rachis width	Rachis hairiness
IM	210	I	IV	IV	MP	123	IV	IV	*D*	VM	7	V	IV	*D*
	213	IV	*D*	V		129	IV	V	*ID*		12	IV	I	*ID*
	219	V	*ID*	*ID*		130	I	IV	V		15	IV	IV	*D*
	225	V	V	I		131	I	V	*ID*		17	IV	V	V
	226	IV	IV	*ID*		134	IV	IV	V		22	V	IV	*ID*
	227	V	V	I		137	IV	IV	*D*		23	V	V	*ID*
	234	V	IV			139	*D*	I	I		28	I	I	*ID*
	238	I	*ID*	I		140	I	I	IV		29	IV	IV	*ID*
	241	V	V	*ID*		142	V	V	*D*		32	I	I	*D*
	242	V	V	V		143	V	I	I		34	IV	I	*ID*
	247	*ID*	IV	IV		144	V	IV	V		36	IV	V	V
	249	IV	*ID*	IV		145	*ID*	I	IV		37	IV	V	*D*
	250	I	IV	I		146	IV	V	V					
	251	I	IV	I		154	I	I	IV					
	252	V	IV	*ID*		160	IV	IV	IV					
	253	*ID*	IV	V		165	V	V	V					
	255	IV	V	*ID*										
	259	IV	*ID*	V										

vulgare-like plants from plump seeds (see Table I). The IM section of the table shows that although there were fewer *vulgare*-like plants from shrunken seeds (18) than from plump seeds (21), the former showed a sprinkling of D or ID for *all* of the three characters under consideration. The MP section shows D or ID for two of the characters concerned but none of the plants had *persicum* resemblance for rachis width. The VM section of the table shows a large proportion of D and ID for rachis hairiness but no D or ID for the other two characters.

Durum-vulgare Character Relationships

The discovery that the *vulgare*-like plants from plump seeds were neither D nor ID for three characters in each cross immediately brought up the question of the frequency of the various interspecific character relations As both the IM and the VM crosses were being used in a breeding program they were utilized for a study of this question. Random lots of F_2 plants grown from plump and from shrunken seeds of each cross were examined and their degree of resemblance to the parental varieties was recorded for each of a number of morphologic characters. The various character relations were analyzed, first on the basis of both V and IV indicating *vulgare*ness and both D and ID representing resemblance to the 14-chromosome parent, and finally on the basis of combinations of V with D, only. The preliminary analysis of the population from plump seeds of the Iumillo × Marquis cross is given in Table III.

The table was prepared as follows: Plant No. 1 was considered for the relation between spike form and each of the twelve other characters.

TABLE III

THE FREQUENCY DISTRIBUTION OF COMBINATIONS OF V OR IV WITH D OR ID FOR ALL POSSIBLE TWO-CHARACTER RELATIONS IN THE 102 *durum* × *vulgare* F_2 PLANTS FROM *plump* SEEDS

Character	Spike form	Spike compactness	Stem hollowness	Stem thickness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Beak character	Keel prominence	Brush character	Seed character
Spike form													
Spike compactness	7												
Stem hollowness	5	7											
Stem thickness	15	21	13										
Rachis width	18	20	16	12									
Rachis hairiness	9	11	13	19	17								
Shoulder width	13	12	10	19	13	14							
Glume l-w ratio	11	19	11	20	18	20	11						
Keel sharpness	10	17	16	21	24	19	24	25					
Beak character	25	37	27	26	23	37	31	25	29				
Keel prominence	11	13	13	25	21	14	17	18	11	29			
Brush character	17	22	18	18	14	15	18	17	21	20	21		
Seed character	13	20	17	22	20	19	15	10	20	24	21	18	
Average	13	17	14	19	18	17	16	17	20	28	18	18	18

This plant was ID for spike form. It was V or IV for rachis width and three other characters. In each case a mark was made in the square where the line or column for spike form met the column or line for the other character, following to some degree the method used in filling in an ordinary correlation surface. The next step was to consider the spike compactness of Plant No. 1 in its relations, and so on for all the characters of Plant No. 1. Each of the 101 other plants was considered in the same way. To prevent duplication of numbers throughout the table all relations were taken from D or ID to V or IV.

Table III demonstrates that *all* of the different two-character combinations of V or IV with D or ID occurred in this random population of only 102 F_2 plants. Beak character showed a high average number of combinations. Stem hollowness and spike form showed only half as many combinations. The other characters were more or less intermediate in this respect.

TABLE IV

COMBINATIONS OF V (*vulgare*NESS) WITH D (*durum*NESS) FOR ALL POSSIBLE TWO-CHARACTER RELATIONS IN THE 102 *durum* × *vulgare* F_2 PLANTS FROM *plump* SEEDS

Character	Spike form	Spike compactness	Stem hollowness	Stem thickness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Beak character	Keel prominence	Brush character	Seed character
Spike form													
Spike compactness	2												
Stem hollowness	0	0											
Stem thickness	7	11	4										
Rachis width	6	7	0	1									
Rachis hairiness	4	5	2	10	4								
Shoulder width	3	3	0	8	3	4							
Glume l-w ratio	2	7	2	10	2	6	2						
Keel sharpness	2	6	3	8	4	5	5	2					
Beak character	13	21	8	10	10	20	11	8	12				
Keel prominence	0	3	2	10	3	3	3	3	2	7			
Brush character	7	10	5	6	4	4	6	6	4	9	5		
Seed character	1	2	0	2	1	4	1	0	0	3	2	1	
Averages	3.9	6.5	2.2	7.3	3.8	5.9	4.1	4.2	4.4	11.0	3.6	5.6	1.4

If, instead of taking V and IV to represent *vulgare*ness and D and ID to represent *durum*ness, only combinations of V with D, that is, full resemblances to the parental species, are taken into account, relatively few combinations are obtained. The results for all possible two-character combinations of V and D in the Iumillo × Marquis plants from plump seeds are given in Table IV. The numbers in the table are small, as would be expected. In eight cases no plant had the combination. Such a result could occur by chance. However, seven of these eight cases occurred with respect to the relationships of only two characters, stem hollowness and seed character, with the other characters. It is evident that for some character pairs combinations of V with D occurred much less frequently than they did for other character pairs.

TABLE V

COMBINATIONS OF V (*vulgare*NESS) WITH D (*durum*NESS) FOR ALL POSSIBLE TWO-CHARACTER RELATIONSHIPS IN THE 55 *durum* × *vulgare* F_2 PLANTS FROM *shrunken* SEEDS

Character	Spike form	Spike compactness	Stem hollowness	Stem thickness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Beak character	Keel prominence	Brush character	Seed character
Spike form													
Spike compactness	0												
Stem hollowness	0	1											
Stem thickness	3	6	1										
Rachis width	0	1	0	1									
Rachis hairiness	4	7	4	9	2								
Shoulder width	1	1	1	1	1	3							
Glume l-w ratio	4	9	0	2	0	4	1						
Keel sharpness	1	2	1	4	1	6	1	5					
Beak character	8	15	3	10	6	10	3	4	9				
Keel prominence	0	1	1	4	2	5	1	3	0	6			
Brush character	4	4	3	6	3	6	2	6	3	7	1		
Seed character	1	1	0	1	0	2	1	0	2	2	0	4	
Averages	2.2	4.0	1.3	4.0	1.4	5.2	1.4	3.2	2.9	6.9	2.0	4.1	1.2

The results on two-character combinations of D and V in the plants from shrunken seeds appear in Table V. Here we find the non-appearance of 12 combinations. The total population was only 55 plants or roughly half of that of the plants from plump seeds, consequently the percentage of absent combinations is lower in the plants from shrunken seeds. Here as in Table IV the distribution of zeros in the table is not fortuitous. Seven of the 11 cases occurred with respect to the relationships of stem hollowness and seed character with the other characters, as was the case in the results in Table IV. Four of the 11 misses occur in the spike form column. Going back to Table IV we find two of the eight misses in the spike form column. The relative frequencies of combinations in Tables IV and V are very similar, the lowest frequencies being for stem hollowness and seed character and the highest for beak character in both sets of figures. There appears to be no essential difference between the populations from shrunken and plump seeds with respect to the frequency of occurrence of different character combinations.

Dicoccum-vulgare Character Relationships

Data on the cross Vernal emmer × Marquis were studied in the same manner as for the *durum* × *vulgare* cross. Before discussing the character combinations in plants from shrunken and from plump seeds, it is of interest to examine the combinations in a random population without regard to endosperm development. Table VI shows the combinations of V (*vulgare*ness) with D (*dicoccum*ness) for all possible two-character relationships in a random population of 276 F_2 plants. All combinations were represented excepting one (seed character with spike form) and it is probable that the absence of that combination is a matter of chance, since several other combinations are represented only once.

TABLE VI

THE FREQUENCY DISTRIBUTION OF THE COMBINATIONS OF V (*vulgare*NESS) WITH D (*dicoccum*NESS) FOR ALL POSSIBLE TWO-CHARACTER RELATIONSHIPS IN A RANDOM POPULATION OF 276 F_2 PLANTS OF THE CROSS VERNAL EMMER × MARQUIS

Character	Rust reaction	Spike form	Spike compactness	Stem hollowness	Rachis articulation	Spikelet adherence	Rachis width	Stem thickness	Glume adherence	Rachis hairiness	Shoulder width	Shoulder shape	Glume l-w ratio	Keel sharpness	Awning	Seed character
Field rust reaction																
Spike form	6															
Spike compactness	40	11														
Stem hollowness	15	3	9													
Rachis articulation	7	4	14	8												
Spikelet adherence	30	6	61	14	8											
Rachis width	21	7	11	2	13	20										
Stem thickness	44	9	21	4	12	21	2									
Glume adherence	31	6	40	12	4	1	18	16								
Rachis hairiness	19	6	10	5	14	39	13	31	25							
Shoulder width	25	14	19	11	18	42	6	14	36	14						
Shoulder shape	53	13	43	17	18	18	11	9	7	37	27					
Glume l-w ratio	18	4	25	4	10	26	1	8	21	14	3	21				
Keel sharpness	47	12	31	12	11	15	13	14	5	31	41	19	25			
Awning	71	35	61	30	21	43	25	17	32	46	36	27	29	38		
Seed character	1	0	25	2	3	7	8	10	6	12	13	17	3	17	25	
Averages	27	9	26	9	10	22	11	14	16	20	20	21	13	21	34	10

Table VII gives the frequency distribution for the various two-character combinations of D with V which were found in a random population of 102 F_2 plants from plump seed of the VM cross. There were 21 missing combinations of which 11 involved spike form and stem thickness, but none involved keel sharpness and awning, and only one was in the spike compactness column.

TABLE VII

COMBINATIONS OF V (*vulgare*NESS) WITH D (*dicoccum*NESS) FOR ALL POSSIBLE TWO-CHARACTER RELATIONSHIPS IN THE 46 *dicoccum* × *vulgare* F_2 PLANTS FROM *plump* SEEDS

Character	Spike form	Spike compactness	Stem hollowness	Rachis articulation	Rachis width	Stem thickness	Glume adherence	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Awning
Spike form												
Spike compactness	1											
Stem hollowness	0	0										
Rachis articulation	0	2	0									
Rachis width	0	1	0	1								
Stem thickness	0	2	1	0	0							
Glume adherence	2	4	2	0	3	1						
Rachis hairiness	0	1	0	0	0	1	3					
Shoulder width	1	3	0	2	1	0	3	1				
Glume l-w ratio	0	2	0	1	1	0	1	0	0			
Keel sharpness	2	7	3	2	2	2	2	6	6	3		
Awning	4	17	10	6	6	2	4	11	8	7	[illegible]	
Averages	0.8	3.3	1.3	1.2	1.3	0.8	2.1	1.9	2.1	1.3	3.5	6.8

In Table VIII, results are given for the plants from shrunken seeds. There were 18 missing combinations, instead of about 24 which would be expected on the basis of the results in Table VII. Another difference between the results in Tables VII and VIII is the greater frequency of combinations involving glume adherence, shoulder width, keel sharpness and awning, in the material from plump seeds, and of combinations with stem thickness in the plants from shrunken seeds. Considering the 18 misses, they involved principally the characters, spike form, stem thickness, shoulder width and glume length-width ratio, but not spike compactness; and only one was concerned with awning. The populations from plump and shrunken seeds may be said to agree less well, as to the frequency of the different character combinations, than did the comparable populations of the IM cross.

TABLE VIII

COMBINATIONS OF V (*vulgare*NESS) WITH D (*dicoccum*NESS) FOR ALL POSSIBLE TWO-CHARACTER RELATIONSHIPS IN THE 39 *dicoccum* × *vulgare* F_2 PLANTS FROM *shrunken* SEEDS

Character	Spike form	Spike compactness	Stem hollowness	Rachis articulation	Rachis width	Stem thickness	Glume adherence	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Awning
Spike form												
Spike compactness	1											
Stem hollowness	1	3										
Rachis articulation	0	2	1									
Rachis width	1	4	1	1								
Stem thickness	1	7	1	0	0							
Glume adherence	0	5	1	0	1	1						
Rachis hairiness	1	3	2	1	0	4	4					
Shoulder width	0	2	1	0	0	2	2	0				
Glume l-w ratio	1	4	1	1	0	0	0	3	1			
Keel sharpness	0	2	2	0	1	0	0	3	2	1		
Awning	2	5	4	2	3	2	0	4	1	2	1	
Averages	0.7	3.2	1.5	0.7	1.0	1.5	1.3	2.1	0.9	1.3	1.0	2.2

Discussion of Results

From the plant breeding standpoint it is important to know, first, if certain character combinations are unobtainable or only rarely found in F_2 populations of inter-specific wheat crosses and, second, whether the lack of these character combinations is linked up with the degree of development of the F_2 seeds.

Frequency of Inter-specific Character Combinations

The most extensive data on the frequency of different D-V character combinations appears in Table VI where the results on 276 F_2 plants of the cross Vernal emmer × Marquis are given. The various combinations showed a wide range in the frequency of their occurrence, seven appearing less than three times each and five others being represented more than 46 times each. The following tabulation gives the frequency distribution of all the combinations:

	Combination frequencies								
	0	1–2	3–5	6–9	10–15	16–23	24–34	35–46	47 up
Number of combinations	1	6	11	17	28	20	18	14	5

Some combinations occurred frequently and others rarely. On the whole, the combinations which occurred most frequently, such as the keel sharpness of one species with the rust reaction of the other, or the spikelet adherence of one species with the spike compactness of the other, are usually considered to be fully as unusual inter-specific combinations as those which appeared rarely, such as the keel sharpness of one species with the glume adherence of the other.

Some characters showed a relatively small tendency to combine inter-specifically with others, namely, spike form and stem hollowness, whereas other characters showed a large tendency toward such combinations, *viz.*: spike compactness, spikelet adherence and rust reaction.

One combination which is economically desirable, that of stem rust resistance with *vulgare* seed character, occurred only once in the whole population. It is easy to see that to add various other characters to this combination might require a very large population.

In practical plant breeding, some of the characters studied in the VM cross could be ignored in selection work as it is unimportant whether they be present in the V, D, or an intermediate condition. Examples are rachis hairiness, shoulder shape and awning. Comparatively few of the characters are necessary to have in the D or V condition. Exceptions are rust reaction and seed character, respectively. The varying frequency of combinations of *vulgare*-ness with *dicoccum*ness for different character pairs should, therefore, be regarded as a guide to the breeder rather than as a detriment to his work. Where he desires combinations which are infrequent, he should use very large populations from which to select.

It is of interest to note that some character combinations were absent from both groups of plants studied in the Iumillo × Marquis cross. The total number of plants was 157 and five combinations were missing. Inspection of the two sets of data shows three combinations which are represented only once in each population. A further random reduction in the size of the populations would be expected to change some of these frequencies of one to misses, and an increase in the sizes of the populations would, by the same reasoning, supply some of the missing combinations. This expectation is fortified by the fact that the distribution for frequency of combination is normal for each group of plants, the only real difference being the natural one that the distribution for the 102 plants from plump seeds has a higher mean than the one for the 55 plants from shrunken seeds. This is shown by the following tabulation:

	Combination frequencies				
	0	1 and 2	3 to 5	6 to 9	10 and over
Table IV data	8	18	24	16	12
Table V data	12	30	20	13	2

Furthermore, there is no reason to expect that a population of several hundred plants would not fill in the missing combinations, as occurred in the case of the VM random population of 276 plants.

Discussion of the frequency of combinations found in the small VM populations from plump and shrunken seeds is not necessary as the situation is covered by the foregoing discussions on the large random VM population and the IM populations.

Relation of Seed Plumpness to Character Combinations

A detailed study of all possible two-character relations was made in a *durum* × *vulgare* and a *dicoccum* × *vulgare* cross to ascertain whether plants from shrunken seeds furnished *any* two-character combinations not obtainable in plants from plump seeds. In 1930 Thompson (9) concluded that the shrunken seeds in inter-specific wheat crosses were much more valuable to the practical breeder than the plump seeds and should receive special care. In 1934 Harrington and Marshall (3), in a study of three inter-specific wheat crosses, concluded that no special attention need be paid to the shrunken seeds as they did not appear to possess character combinations unobtainable in plants from plump seeds.

The results of the *durum* × *vulgare* study presented in Tables IV and V show eight combinations missing in the 102 plants from plump seeds and 12 in the 55 plants from shrunken seeds. This difference slightly favors the plants from shrunken seeds (on the basis of 8 misses in 102 plants 15 could be expected in 55 plants) but does not appear to have significance. Any possible significance is detracted from by the fact that the 102 plants from plump seeds furnished 7 combinations not present in the plants from shrunken seeds. (Spike compactness with spike form, rachis width with spike form, glume length-width ratio with stem hollowness, glume length-width ratio with rachis width, seed character with rachis width, keel prominence with keel sharpness and seed character with keel prominence), whereas plants from shrunken seeds furnished only 3 combinations not found in the plants from plump seeds, (stem hollowness with spike compactness, shoulder width with stem hollowness and seed character with keel sharpness).

All of these combinations involve characters of species-differentiating value as has been shown by Percival (6), Watkins (10) and others. Sapehin (7) and Sapehin (8), have shown that it is difficult and probably erroneous to assign more critical species-differentiating value to one of these characters than to others among them as none of them are found exclusively in a given species. Therefore it is reasonable to regard the missing combinations as being of equal importance. The plants from plump seeds actually possessed a larger proportion of combinations missing in the plants from shrunken seeds than did the latter with respect to the former. It follows that the plants from shrunken seeds cannot be said to have supplied combinations unobtainable in the plants from plump seeds.

The *dicoccum* × *vulgare* results presented in Tables VII and VIII show 21 missing combinations in the 46 plants from plump seeds and only 18 in the 39 plants from shrunken seeds. The difference favors the plants from shrunken seeds and is strengthened by the fact that these plants possessed 15 combinations not shown by the plants from plump seeds, whereas the latter showed only 11 combinations not present in the plants from shrunken seeds. This indicates that, in the *dicoccum* × *vulgare* cross, plants from shrunken seeds might furnish a larger range of combinations than plants from plump seeds. However, the difference between the two sets of results is small and probably not significant. The absence of some combinations in Tables VII and VIII as well as in Tables IV and V appears to be due to the small size of the populations. In the much larger population reported in Table VI there is only one combination missing.

Considering the results on both crosses it may be concluded that there is no evidence to show that F_2 plants from shrunken seeds possess character combinations not obtainable in F_2 plants from plump seeds. In fact, the data suggest that the various two-character combinations are as likely to be obtained from one group of plants as from the other, in either cross.

The results of this study indicate that a sufficiently large population should show all of the possible two-character combinations of V and D. It follows that a larger population would be necessary to reveal all possible three-character combinations of V and D, a still larger population to show all possible four-character combinations, and so on. If it may be assumed that the frequency of occurrence of any given combination of *vulgare*ness for certain characters with *dicoccum*ness for other characters depends largely upon the size of population, then the results presented in Tables IV, V, VII and VIII indicate no appreciable superiority of plants from shrunken seeds over plants from plump seeds.

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